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Chemosystematic investigations of irregular diterpenes in *Anisotome* and related New Zealand Apiaceae

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Abstract

A chemosystematic HPLC–UV and HPLC–MS investigation of New Zealand members of the Apiaceae was performed. Diterpenes were identified and quantified in methanolic extracts from subaerial parts of 28 taxa and 54 samples of *Aciphylla*, *Anisotome, Apium, Gingidia, Lignocarpa, Oreomyrrhis*, and *Scandia*. Six diterpenes (**1**–**2**, **4**–**7**) and four polyacetylenes (**8**–**11**) were identified. The known compounds were the diterpenes anisotomenoic acid **1**, anisotomene-1-ol **2**, 16-acetoxyanisotomenoic acid **4** and anisotomene-1,12-diol **5**; and the polyacetylenes falcarinol **8**, falcarindiol **9**, (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol **10**, and (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol 1-acetate **11**. New irregular diterpenes 13,14-dihydroanisotom-12*E*-ene-1,14-diol **6** and 14-methoxy-13,14-dihydroanisotom-12*E*-ene-1-ol **7** were isolated from *A. haastii*. Isomers of the new semi-synthetic diterpene 16-hydroxyanisotomenoic acid **3** were detected in extracts of *Anisitone flexuosa*. Structure elucidation was performed by HR mass spectrometry and 1D and 2D NMR spectroscopy. In crude extracts, compounds were identified by their HPLC retention times and their on-line HPLC–UV and MS spectra. Anisotomene diterpenes occurred in eight out of 16 species of the genus *Anisotome*, but were not detected in any of the other genera. In contrast, polyacetylenes were present in all the genera investigated.

Keywords: *Anisotome*; *Aciphylla*; *Gingidia*; *Lignocarpa*; *Scandia*; Apiaceae; Chemosystematics; Diterpenes; Polyacetylenes

1. Introduction

The genus *Anisotome* (Apiaceae, tribe Apieae) comprises in its current delimitation 16 species, with 12 species endemic to New Zealand's main islands (North, South and Stewart), three species endemic to New Zealand's subantarctic islands and one Tasmanian endemic species (Allan, 1961; Dawson, 1961; Webb, 1986). Details of full scientific names and distributions are given in Table 1. Many New Zealand Apiaceae, belonging to the subfamily Apioideae (except the members of the distinct genus *Aciphylla*), were formerly included on the basis of superficial morphological similarities into the Northern Hemisphere genera *Angelica* and *Ligusticum* (Hooker, 1864; Cheeseman, 1925). More detailed morphological studies revealed that the New Zealand taxa are only distantly related to these genera. Today the species at hand are grouped into Southern Hemisphere genera *Aciphylla*, *Anisotome*, *Gingidia*, *Lignocarpa* and *Scandia* (Dawson, 1961, 1967a,b, 1968, 1971) but delimitations of these genera are still controversial (Webb, 1986; Mitchell et al., 1998; Parsons et al., 1998). RDNA sequence data (Mitchell et al., 1998) are congruent with the monophyly of *Anisotome* as delimited by Dawson (1971). However, only three species of *Anisotome* (*A. aromatica*, *A. deltoidea* and *A. filifolia*) were included in this phylogenetic study.

Anisotome flexuosa contains high levels of anisotomenoic acid **1** and anisotomen-1-ol **2** (Fig. 1), which represent a new class of irregular diterpenes proposed to arise from head to head coupling of two monoterpene moieties (van

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Table 1
Full scientific names and distribution areas of the investigated species (Allan, 1961; Dawson, 1961; Webb, 1986; Parsons et al., 1998)

Taxon	Distribution
Aciphylla aurea W.R.B.Oliv.	NZ South Island
Ac. dieffenbachii (F.Muell.) Kirk	Chatham Islands
Ac. scott-thomsonii Cockayne & Allan	NZ South Island
Ac. similis Cheeseman	NZ South Island (Canterbury, Westland)
Ac. subflabellata W.R.B.Oliv.	NZ South Island
Anisotome acutifolia (Kirk) Cockayne	Snares and Solander Islands
A. antipoda Hook.f.	Antipodes, Auckland, and Campbell Islands
A. aromatica Hook.f.	NZ main islands
A. brevistylis (Hook.f.) Poppelwell	NZ South Island (Otago)
A. capillifolia (Cheeseman) Cockayne	NZ South Island (Otago, Southland)
A. cauticola J.W.Dawson	NZ South Island (Otago)
A. deltoidea Cheeseman	NZ South Island
A. filifolia (Hook.f.) Cockayne & Laing	NZ South Island
A. flexuosa J.W.Dawson	NZ South Island and Stewart Island
A. haastii (F.Muell. ex Hook.f.) Cockayne & Laing	NZ South Island and Stewart Island
A. imbricata (Hook.f.) Cockayne var. *imbricata*	NZ South Island (Otago)
A. imbr. var. *prostrata* J.W.Dawson	NZ South Island
A. lanuginosa (Kirk) J.W.Dawson	NZ South Island
A. latifolia Hook.f.	Antipodes and Campbell Islands
A. lyallii Hook.f.	NZ South, Stewart Island and Solander Island
A. pilifera (Hook.f.) Cockayne & Laing	NZ South Island
A. procumbens (F.Muell.) C.J. Webb	Tasmania
Apium prostratum Vent.	Widespread in the southern hemisphere
Gingidia decipiens (Hook.f.) J.W.Dawson	NZ South Island
G. montana (J.R.Forst. & G.Forst.) J.W.Dawson	Australia (New South Wales), NZ North Island and South Island
Lignocarpa carnosula (Hook.f.) J.W.Dawson	NZ South Island
Oreomyrrhis colensoi Hook.f.	NZ main islands and Chatham Islands
Scandia geniculata (G.Forst.) J.W.Dawson	NZ North Island and South Island

Klink et al., 1999). We now report analyses of all taxa of *Anisotome*, some New Zealand taxa of the related genera *Aciphylla*, *Gingidia*, *Lignocarpa* and *Scandia*, and one species of both of the more distantly related genera *Apium* and *Oreomyrrhis* (Table 1). Subaerial parts were chosen for this investigation to avoid complications with chlorophyll in the HPLC analysis. However, diterpenes are also—albeit in usually smaller amounts—detectable in the leaves of the anisotomene containing taxa.

In addition to *A. flexuosa*, five other species of *Anisotome* have previously been investigated phytochemically. Essential oils from *A. antipoda* contained monoterpenes, and oils from *A. latifolia* contained sesquiterpenes and phenylpropanoids (van Klink and Perry, 1998). Further investigations of *A. flexuosa* yielded **4** and *A. haastii* yielded **5**, both diterpenes with the anisotomene skeleton (van Klink et al., 2000). *A. pilifera* yielded, in addition to **1** and **2**, a new highly oxygenated sesquiterpene (Zidorn and Perry, 2002a). *A. lyallii* contained a rare phenylpropanoid, and three new highly oxygenated sesquiterpenes (Zidorn and Perry, 2002b).

The only previous phytochemical investigations of the other New Zealand Apiaceae revealed the presence of β-sitosterol in *Aciphylla colensoi* (Cambie and Parnell, 1969), a number of polyacetylenes in *Ac. scott-thomsonii* (Perry et al., 2001) and the occurrence of furanocoumarins in *Apium prostratum* (Diawara et al., 1993, 1994; Trumble et al., 2000). To the best of our knowledge no members of the genera *Gingidia*, *Lignocarpa* and *Scandia* have been investigated phytochemically.

The aims of the present study were: (a) to search for compounds that might give clues to the biosynthetic steps in the formation of the anisotomenes; (b) to determine whether anisotomenes are useful chemosystematic markers of particular *Anisotome* species, and/or delimit the genus from related genera; and (c) to establish LC–MS systems for the analysis of anisotomene-type diterpenes and polyacetylenes in crude extracts.

2. Results

2.1. New anisotomenes

HPLC traces of all samples of *A. flexuosa* showed, besides peaks for known anisotomenes **1**, **2** and **4**, an additional minor peak. The on-line LC-mass spectrum of

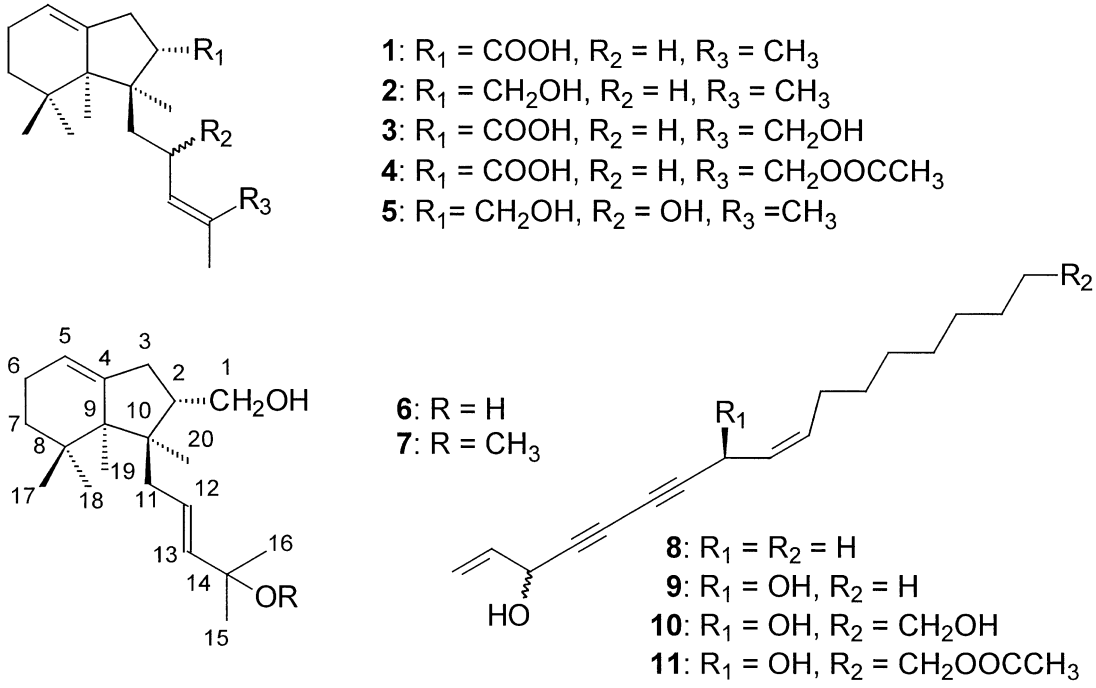

Fig. 1. Diterpenes (**1**–**7**) and polyacetylenes (**8**–**11**) isolated from *Anisotome* and *Aciphylla*.

this peak showed signals congruent with a molecular ion of 320 and a molecular formula of $C_{20}H_{32}O_3$. This is the mass to be expected for a hydroxy–anisotomenoic acid derivative such as 16-hydroxyanisotomenoic acid **3**, the deacetylated form of compound **4**. This new compound was synthesized from 16-acetoxyanisotomenoic acid **4** by saponification and identified as **3** by ^{1}H NMR (Table 2) and ^{13}C NMR (Table 3) spectroscopy and mass spectrometry. Compound **3** had the same on-line MS fragmentation pattern as the minor peak in *A. flexuosa*, but co-chromatography revealed that **3** does not occur as a natural compound. But at least two different compounds showing the same on-line MS fragmentation pattern but slightly different retention times in HPLC system 2 (36.0 and 38.3 instead of 40.3 min) occur as minor compounds in *A. flexuosa*, *A. aromatica*, *A. imbricata* and *A. lyallii*, suggesting the existence of other hydroxyanisotomenoic acid derivatives.

Known compounds (van Klink et al., 1999, 2000) **2** (3.45%) and **5** (0.08%) as well as the new anisotomene-derivatives **6** (0.26%) and **7** (0.02%) were isolated from the methanol extract of subaerial parts of *A. haastii*. HR EIMS data of **6** indicated a molecular formula of $C_{20}H_{32}O$, but ^{13}C NMR data (Table 3) showed two oxygenated carbon signals and the IR spectrum showed OH signals. Therefore, we proposed a molecular formula of $C_{20}H_{34}O_2$ with a facile loss of H_2O in EIMS. ^{1}H and ^{13}C NMR data (Tables 2 and 3) in combination with HSQC and HMBC data revealed that **6** had an anisotomene-type carbon skeleton. Spectra of **6** and **2** (van Klink et al., 1999) were in most parts almost superimposable and differed only in the signals attributable to the side-chain. The occurrence of an additional vinylic proton [H-12, δ_H 5.64, *ddd* (15.5, 7.5, 5.5)] in the ^{1}H NMR spectrum and HMBC crosspeaks from that proton, and from the signals of the protons assignable to the methyl groups in positions 15 and 16 to a quaternary carbon signal at δ_C 70.5 ppm indicated that the side-chain double-bond was in position 12 instead of 13, and that C-14 was substituted with a hydroxy-group. The *E*-configuration of this double bond was shown by the coupling between protons 12 and 13 (15.5 Hz). Thus, **6** is 13,14-dihydroanisotom-12*E*-ene-1,14-diol.

Compound **7** gave EIMS, ^{1}H and ^{13}C NMR data (Tables 2 and 3) very similar to those of **6**. The only differences were: (a) the occurrence of additional signals assignable to a methoxyl group (δ_H 3.13 3H, *s*; δ_C 50.2); (b) downfield shifts for the signals assigned to C-12 and C-14 (2.4 and 4.5 ppm, respectively); (c) upfield shifts for the signals assignable to C-13, C-15 and C-16 (2.6, 4.2, 3.1 ppm, respectively), and (d) less pronounced downfield shifts for the signals assignable to H-12, H-13 (0.05 and 0.17 ppm), respectively. These data were best explained by a methoxyl-group at C-14. A HMBC crosspeak between the protons of the methoxy-group and C-14 confirmed this assumption and therefore **7** was identified as 14-methoxy-13,14-dihydroanisotom-12*E*-ene-1-ol. Methyl ether **7** may be an artefact of oxidative addition of the extraction solvent methanol to alcohol **2**. However, **7** was not detected in all the extracts containing high levels of **2**. Furthermore, methanolic stock solutions of **2** showed no formation of **7** after three months.

Table 2
^{1}H NMR spectral data of irregular diterpenes **3**, **6** and **7**[a]

Position	3	6	7
1		3.66 1H, *dd* (10.5, 6.0)	3.66 1H, *dd* (10.5, 6.0)
		3.46 1H, *dd* (10.5, 8.0)	3.52 1H, *dd* (10.5, 8.5)
2	2.65 1H, *t* (9.0)	2.02 1H, *m**	2.01 1H, *m**
3	2.77 1H, *m**	2.27 1H, *m**	2.26 1H, *m**
	2.16 1H, *m**	2.04 1H, *m**	2.10 1H, *m**
5	5.42 1H, *d* (6.0)	5.37 1H, br *d* (5.5)	5.39 1H, *m*
6	2.16 2H, *m**	2.03 1H, *m**	2.02 1H, *m**
		1.94 1H, *m**	1.94 1H, *m*
7	1.59 1H, *m**	1.50 1H, *m**	1.51 1H, *m*
	1.25 1H, *m**	1.28 1H, *m**	1.27 1H, *m*
11	1.70 1H, *m**	2.25 1H, *dd* (13.0, 4.5)	2.28 1H, *dd* (12.5, 7.0)
	1.32 1H, *m**	2.01 1H, *m**	2.08 1H, *dd* (12.5, 7.5)
12	2.05 1H, *m**	5.64 1H, *ddd* (15.5, 7.5, 5.5)	5.59 1H, br *dt* (15.5, 7.0)
	1.97 1H, *m**		
13	5.26 1H, br *t* (6.5)	5.59 1H, *d* (15.5)	5.42 1H, br *d* (15.5)
15	1.78 3H, *d* (1.5)	1.27 3H, *s*	1.24 3H, *s*
16	4.17 1H, *d* (12.0)	1.29 3H, *s*	1.24 3H, *s*
	4.13 1H, *d* (12.0)		
17	1.05 3H, *s*	1.06 3H, *s*	1.07 3H, *s*
18	0.93 3H, *s*	0.92 3H, *s*	0.92 3H, *s*
19	1.01 3H, *s*	0.90 3H, *s*	0.92 3H, *s*
20	1.12 3H, *s*	1.04 3H, *s*	1.03 3H, *s*
MeO			3.13 3H, *s*

[a] Measured in $CDCl_3$ at 500 MHz, coupling constants *J* (Hz) are given in parentheses. *Signals not resolved.

2.2. Comparative HPLC investigations

Collections were made of 28 taxa and 54 samples of *Aciphylla* (5 taxa/6 samples), *Anisotome* (17/41), *Apium* (1/1), *Gingidia* (2/3), *Lignocarpa* (1/1), *Oreomyrrhis* (1/1), and *Scandia* (1/1). Most of the taxa were represented by one sample only. In contrast, *A. aromatica* and *A. flexuosa* were represented by a number of collections. *A. flexuosa* is a common species in the sub-alpine zone of the South Island and was therefore easily accessible. A greater number of samples from *A. aromatica* was investigated to include all available morphological variants of this species. Methanolic extracts of the subaerial parts were analyzed by HPLC with UV detection (all 54 samples) and by HPLC–MS (at least one sample from each taxon). Absence or presence of compounds **1**–**11** was judged on the retention times and the on-line mass spectra of the observed peaks in comparison with the reference compounds.

Anisotomenoic acid derivatives (**1**, **3**–**4**) anisotomen-1-ol derivatives (**2**, **5**–**7**), and polyacetylenes (**8**–**11**) showed notable differences in their fragmentation patterns using APCI in the positive mode and CH_3OH as the organic mobile phase. Anisotomenoic acid derivatives gave strong $[M+H]^+$ and $[M-H_2O+H]^+$ signals as well as adducts of these ions with one or two molecules of CH_3OH. In contrast, anisotomenol derivatives showed no adducts with CH_3OH and all gave the most intense signal for $[M-H_2O]^+$, except for the methoxy-derivative **7** (most intense signal $[M-CH_3OH+H]^+$). In the on-line mass spectra of the polyacetylenes a $[M+H]^+$ signal was observed only for falcarinol **8**. The most intense signal of **8** was for $[M-H_2O+CH_3OH]^+$, while **9**–**11** showed most

Table 3
^{13}C NMR spectral data of irregular diterpenes **3**, **6** and **7**[a]

Position	3	6	7
1	180.1	66.4	66.8
2	49.3	45.1	45.9
3	32.4	34.1	33.8
4	144.6[b]	145.4[b]	145.1[b]
5	117.6	116.5	116.8
6	21.9	22.0	22.0
7	36.0[b]	36.2[b]	36.2[b]
8	36.7	36.6	36.7
9	50.5[b]	50.6[b]	49.7[b]
10	53.2[b]	49.9[b]	50.5[b]
11	41.6	44.4	44.9
12	23.2	124.5	127.9
13	133.9	141.1	138.5
14	129.1	70.5	75.0
15	21.3	30.1	25.9
16	61.7	29.0	25.9
17	28.7[b]	28.2[b]	28.3[b]
18	25.8[b]	26.0[b]	26.0[b]
19	19.8[b]	20.9[b]	20.9[b]
20	17.4[b]	16.9[b]	17.0[b]
MeO			50.2

[a] Measured in $CDCl_3$ at 125 MHz.
[b] Signals broadened due to conformational exchange.

intense signals for $[M-2H_2O+CH_3OH]^+$. All polyacetylenes showed other main signals at $[M-xH_2O+yCH_3OH+H]^+$, with x = 0, 1 or 2 and y = 0, 1, 2 or 3. In the mono-oxygenated compound **8** only one hydroxy group could be split off as H_2O ($x=0$ or $x=1$). Diols **9**–**10** and triol **11** exhibited signals with $x=0$, $x=1$ and $x=2$. A positive correlation was observed between the number of H_2O molecules split off and the number of CH_3OH molecules added. The internal standard *N*-phenylundecanamide was characterized by on-line mass signals at *m*/*z* 262 $[M+H]^+$ (100), 294 $[M+CH_3OH+H]^+$ (92), and 523 $[2M+H]^+$ (12).

Known compounds **1**–**2**, **4**–**5** and **9**–**11** were identified with authentic reference substances isolated from *A. flexuosa* (van Klink et al., 1999, 2000), *A. haastii* (van Klink et al., 2000), *A. pilifera* (Zidorn and Perry, 2002a) and *Aciphylla scott-thomsonii* (Perry et al., 2001), respectively. Falcarinol **8** was isolated from subaerial parts of *A. latifolia* and identified by ^1H and ^{13}C NMR spectroscopy and comparison of the data with the literature (Gafner et al., 1989). New compounds **3**, **6** and **7** were isolated and identified as described above. The results of the comparative HPLC analyses are summarized in Table 4.

Anisotomenes were detected in eight of the 16 species of *Anisotome*, namely *A. aromatica*, *A. brevistylis*, *A. filifolia* (only traces), *A. flexuosa*, *A. haastii*, *A. imbricata* var. *imbricata*, *A. imbricata* var. *prostrata*, *A. lyallii*, and *A. pilifera*. No anisotomene-type diterpenes were detected in any samples from the genera *Aciphylla*, *Gingidia*, *Lignocarpa* and *Scandia*, which are closely related to *Anisotome*, nor were anisotomenes detected in samples from the distantly related New Zealand taxa of *Apium* and *Oreomyrrhis*. Polyacetylenes falcarinol **8** and falcarindiol **9**, which seem to be ubiquitous in the Apiaceae (Bohlmann, 1971), were also detected in most of the New Zealand species investigated. The rare C_{18}-polyacetylenes **10** and **11** were found at high levels only in *Aciphylla scott-thomsonii* (Perry et al., 2001) and *Lignocarpa carnosula*, and are therefore not included in Table 4.

3. Discussion

In all the 54 samples of 28 Apiaceae taxa analyzed, only diterpenes of the anisotomene-type were found (Table 4). It is possible that low polarity diterpenes might not have been eluted from the HPLC, but previous GC analyses of the anisotomene-rich species *A. flexuosa* and *A. haastii* did not indicate any less oxidized diterpenes (van Klink et al., 2000). We had hoped to find some other irregular diterpenes, or even sesquiterpenes, such as those reported in a few other Apiaceae species (van Klink et al., 2000). However, it seems that the biosynthetic pathway to the anisotomenes is tightly controlled, with the only variations being the degree of oxidation in compounds **1**–**2** and **4**–**7**.

The distribution of irregular diterpenes in New Zealand Apiaceae is not easily interpreted. Anisotomenes are confined to the genus *Anisotome*, but not all investigated samples of *Anisotome* contained anisotomene-derivatives (Table 4). Notably, none or only traces of anisotomenes were detected in *A. deltoidea* and *A. filifolia*, which Dawson (1961) excluded from the genus on a morphological foundation but later tentatively re-integrated into the genus (Dawson, 1971). Also in the sub-Antarctic species of *Anisotome* (*A. acutifolia*, *A. antipoda*, *A. latifolia*) and the Tasmanian species *A. prostrata* no diterpenes were detectable. Anisotomenes were not detected in samples of mainland New Zealand species *A. capillifolia*, *A. cauticola,* and*A. lanuginosa*. In all investigated populations of *A. aromatica*, *A. flexuosa*, *A. haastii, A. lyallii* and *A. pilifera* anisotomenes were found. In *A. brevistylis* and *A. imbricata* some samples contained considerable amounts of anisotomenes, whereas in other samples these substances were not detectable.

The pronounced intraspecific qualitative and quantitative variations in the anisotomene content could be because these diterpenes have a biological function and their contents change due to environmental factors. The ecological role of anisotomenes is still unknown, but they are present at high levels in some samples, e.g. up to 14 mg/g of **1** in *A. flexuosa*. Bioactivity assays on **1** and **2** revealed some biological activity (van Klink et al., 1999). The available data allow no speculation whether the infraspecific variation in *A. aromatica*, *A. haastii* and *A. imbricata* is related to infraspecific taxa of these species or whether they reflect seasonal and or ecological differences between the investigated samples. In *A. aromatica* the variation in the secondary metabolite spectrum is not confined to anisotomenes but also encompasses other not identified compounds. Some samples show HPLC–UV and on-line mass spectra (*m*/*z* = 435, 417, 399, 317 and 217) for up to eight unknown isomeric compounds (e.g. in sample CZ-000203-7), whereas most other *A. aromatica* collections lack these compounds. *A. aromatica* has been divided into eight varieties by Allan (1961), two of those (*A. flexuosa* and *A. lanuginosa*) have been accorded specific rank by Dawson (1961). *A. aromatica* in the delimitation of Dawson (1961) is still a morphologically variable species and chemical data also indicate that it might be a group of different taxa. Our data are not sufficient to solve that problem. However, the observed chemotypes seem not to coincide with the morphological varieties distinguished by Allan (1961).

In contrast, *A. flexuosa* and *A. lanuginosa*, which were formerly included in the concept of *A. aromatica* (Allan, 1961) are chemically well defined. *A. flexuosa* was the only species containing **4** at high levels, and *A. lanuginosa* was characterized by a so far unidentified compound

Table 4
Distribution of anisotomene-type diterpenes (**1**–**2**, **4**–**7**) and polyacetylenes (**8**–**9**) in New Zealand Apiaceae[a]

Taxon[b]	n[c]	1	2	4	5	6	7	8	9
Anisotome									
A. acutifolia	1	-	-	-	-	-	-	+	+
A. antipoda	1	-	-	-	-	-	-	-	+
A. aromatica	11	(+)/++	−/++	-	-	-	-	−/(+)	−/+
A. brevistylis	2	−/+	-	−/(+)	-	-	-	(+)	(+)
A. capillifolia	1	-	-	-	-	-	-	-	+
A. cauticola	1	-	-	-	-	-	-	-	(+)
A. deltoidea	2	-	-	-	-	-	-	-	−/(+)
A. filifolia	1	(+)	-	-	-	-	-	+	(+)
A. flexuosa	7	++	−/+	−/++	-	-/(+)	-	-	−/(+)
A. haastii	2	(+)/+	+/++	-	(+)/+	(+)/+	−/(+)	-	−/(+)
A. imbricata var. *imbricata*	2	−/++	−/++	-	-	-	-	-	−/(+)
A. imbricata var. *prostrata*	3	−/++	−/+	-	-	-	-	−/(+)	(+)/+
A. lanuginosa	2	-	-	-	-	-	-	-	-
A. latifolia	1	-	-	-	-	-	-	+	++
A. lyallii	1	++	+	-	(+)	(+)	-	-	-
A. pilifera	2	+	+	-	(+)	(+)	-	-	+
A. procumbens	1	-	-	-	-	-	-	-	(+)
Aciphylla									
Ac. aurea	2	-	-	-	-	-	-	-	+/++
Ac. dieffenbachii	1	-	-	-	-	-	-	(+)	++
Ac. scott-thomsonii[d]	1	-	-	-	-	-	-	-	+
Ac. similis	1	-	-	-	-	-	-	(+)	++
Ac. subflabellata	1	-	-	-	-	-	-	(+)	++
Apium									
Ap. prostratum subsp. *prostratum* var. *filiforme*	1	-	-	-	-	-	-	(+)	(+)
Gingidia									
G. decipiens	2	-	-	-	-	-	-	+/++	(+)/+
G. montana	1	-	-	-	-	-	-	+	++
Lignocarpa									
L. carnosula[d]	1	-	-	-	-	-	-	-	+
Oreomyrrhis									
O. colensoi	1	-	-	-	-	-	-	(+)	(+)
Scandia									
S. geniculata	1	-	-	-	-	-	-	(+)	+

[a] Results of HPLC/UV investigations; amounts estimated by ratio of peak areas to areas of *N*-phenylundecanamide as internal standard. ++ Estimated amount >5.0 mg/g dried plant material, + estimated amount 1.0–5.0 mg/g dried plant material, **(+)** estimated amount <1.0 mg/g dried plant material, – not detectable. A table with the actual quantification results including standard deviations is available as supplementary material from the corresponding author.

[b] Nomenclature of New Zealand plants is in accordance with Parsons et al. (1998).

[c] Indicates the number of samples investigated.

[d] These species contained additionally high (++/+) amounts of C_{18}-polyacetylenes (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol (**10**) and (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol 1-acetate (**11**).

[$RT_{HPLC\text{-}system\text{-}1}$ = 45.7 min, $RT_{HPLC\text{-}system\text{-}2}$ = 41.7 min, on-line mass spectrum: *m*/*z* = 419 (6), 401 (84), 361 (16), 319 (100), 301 (76), 261 (96)], which was absent or only present as a trace compound from all other investigated extracts. The on-line mass fragmentation pattern suggested that this compound was another trihydroxygermacradiene hemiterpenic acid diester, isomeric to those found in *A. lyallii* (Zidorn and Perry, 2002b). The specific status of these two taxa as assigned by Dawson (1961) is therefore supported by chemical data.

We have noted elsewhere (Zidorn and Perry, 2002b) that *A. pilifera*, *A. lyallii*, *A. aromatica*, *A. haastii*, both varieties of *A. imbricata* and *A. lanuginosa* were distinguished by the presence of 6,8,11-trihydroxygermacrane-1(10),4-diene derivatives. This pattern of oxygenation has not been reported from any other sources.

Polyacetylenes **8**–**9** were found in most of the samples (Table 4). Polyacetylenes are problematic chemosystematic markers because of their pronounced instability. Differences in the content of polyacetylenes were potentially caused by storage of the plant material before analysis and not necessarily by differing contents of polyacetylenes in the investigated plant samples. We have found that mass spectral fragmentation and clustering patterns observed for the polyacetylenes tend to

follow simple rules, and so might serve in the structure elucidation of polyacetylenic compounds too unstable for isolation and subsequent NMR analysis. Ion formation patterns of the diterpenes are less characteristic but might also give first hints on the occurrence and some structural characteristics of diterpenes in crude extracts.

New Zealand Apiaceae differ from most other members of the family in their tendency to hybridize. Many interspecific and even some intergeneric hybrids have been described (Webb and Druce, 1984). It would be of special interest to investigate such hybrids and in particular hybrids between anisotomene-containing and anisotomene-free taxa. Although the New Zealand genera *Aciphylla*, *Anisotome*, *Gingidia*, *Lignocarpa* and *Scandia* are considered to be closely related on the basis of morphological features and their breeding systems (Webb, 1979), no phytochemical markers to differentiate these genera from other genera of the family were identified. Anisotomenes and sesquiterpenes differentiate some *Anisotome* taxa from others lacking those compounds. However, further molecular biological studies are needed to decide whether this fact implies a monophyletic group within *Anisotome* with the ability to synthesize these compounds.

4. Experimental

4.1. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on a Varian V-500 spectrometer at 500 and 125 MHz, respectively. All spectra were measured in $CDCl_3$ and referenced to solvent residual peaks of $CHCl_3$ at $\delta_H = 7.25$ ppm and solvent peaks of $CDCl_3$ at $\delta_C = 77.0$ ppm.

4.2. Plant material

Full collection data, including voucher codes, are given in the Appendix (Table II). Voucher specimens are preserved in the herbarium of the Plant Extracts Research Unit. Identifications were confirmed by J. Dawson.

4.3. Origin of reference compounds

Compounds **1**–**2** (van Klink et al., 1999), **4**–**5** (van Klink et al., 2000), **9** (Zidorn and Perry, 2002a), and **10**–**11** (Perry et al., 2001) have been isolated from *A. flexuosa*, *A. haastii*, *A. pilifera* and *Aciphylla scott-thomsonii*, respectively, as described previously.

4.4. Synthesis of 16-hydroxyanisotomenoic acid **3**

A sample of **4** (25 mg) was stirred with NaOMe in MeOH (5 ml) for 2 h, the solvent was removed under vacuum and the residue partitioned between water (acidified with HCl) and chloroform. The organic fraction was further purified by semi-preparative (RP18) HPLC using an isocratic mixture of acetonitrile and water acidified with 0.1% formic acid (60:40) at a flow rate of 5.00 ml/min. Peaks were detected at 206 nm and a peak that eluted from the column (Phenomenex Luna 250×10 mm) at 14 min was **3** (5.0 mg). Colorless oil; $[\alpha]_D^{17}$ −34.3° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (4.05); IR (film) ν_{max} 3420 (*br*), 2967, 2933, 2867, 1717, 1589, 1483, 1456, 1394, 1378, 1333, 1278, 1239, 1222, 1172, 1116, 1100, 1072, 956 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; EIMS *m*/*z* 302 (2) $[M-H_2O]^+$; 287 (5) $[M-H_2O-CH_3]^+$; HREIMS *m*/*z* 320.23496 (calc. for $C_{20}H_{32}O_3$, 320.23515) $[M]^+$.

4.5. Isolation of compounds **2**, **5**–**7**

Air-dried subaerial parts (380 g) of *A. haastii* (CZ-000128-1) were ground and exhaustively extracted with MeOH. The residue obtained after evaporating the solvent in vacuo (106 g), was partitioned between MeOH/H_2O and EtOAc. The EtOAc phase was dried in vacuo to yield 42.6 g of residue. The residue was then partitioned between MeOH/H_2O and cyclohexane. The residue of the cyclohexane phase (24.6 g) was fractionated by silica-gel CC using a gradient from CH_2Cl_2 to MeOH. Fractions 1 and 2 yielded 13.1 g of **2**. The dioxygenated compounds **5**–**7** were isolated from fraction 6 (4.5 g) by Sephadex LH-20 CC employing a mixture of MeOH and CH_2Cl_2 (1/1, v/v) as eluant and subsequent silica-gel CC using a gradient from cyclohexane to EtOAc to yield **5** (322 mg) and **6** (993 mg). Fractions containing **7** (891 mg), were further fractionated by silica-gel CC employing a gradient from cyclohexane to EtOAc and consecutive repeated Sephadex LH-20 CC using MeOH as eluant, to yield 59.7 mg of **7**.

4.6. 13,14-Dihydroanisotom-12E-ene-1,14-diol **6**

Compound **6** was obtained as a colorless oil; $[\alpha]_D^{28.4}$ −58.0° (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 203 (3.89); IR (film) ν_{max} 3330 (*br*), 2970, 1458, 1378, 1151, 1014 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; EIMS *m*/*z* 288 (2) $[M-H_2O]^+$; 273 (3) $[M-H_2O-CH_3]^+$; HREIMS *m*/*z* 288.24431 (calc. for $C_{20}H_{32}O$, 288.24431) $[M-H_2O]^+$.

4.7. 14-Methoxy-13,14-dihydroanisotom-12E-ene-1-ol **7**

Compound **7** was obtained as an colorless oil; $[\alpha]_D^{23.5}$ −23.5° (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (3.90); IR (film) ν_{max} 3420 (*br*), 2972, 1652, 1456, 1378, 1071 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 2 and 3; EIMS *m*/*z* 320 (2) $[M]^+$, 305 (20) $[M-CH_3]^+$, 288 (60) $[M-CH_3OH]^+$.

4.8. Isolation of **8**

Air-dried subaerial parts (494 g) from cultivated plants of *A. latifolia* (voucher: CZ-000811-2) were extracted exhaustively with MeOH. The residue of the extract obtained after evaporation in vacuo (77.6 g) was partitioned between MeOH/H_2O and EtOAc. The residue of the EtOAc layer (35.2 g) was fractionated by silica-gel CC using a gradient of cyclohexane, EtOAc and MeOH. Enriched fractions of **8** (960 mg) were finally purified by Sephadex LH-20 CC using MeOH as eluant, yielding 128 mg of **8** (Gafner et al., 1989).

4.9. Extraction for HPLC analyses

Air dried subaerial parts (no investigations were made on the nature—roots or rhizomes—of the investigated plant parts in the different species, also no separation in main and side-roots was performed) of the plants were ground with a Breville CG2 coffee and spice grinder. Then 500 mg of plant material was mixed with 10.0 ml of a methanolic stock solution containing 0.200 mg/ml of *N*-phenyl-undecanamide (Perry et al., 1996) as internal standard and extracted three times with 25 ml of methanol for 7.5 min with an IKA-25 Ultraturrax apparatus at 24,000 cycles/min. Extracts were combined and brought to dryness in vacuo. The residue was dissolved in 4.00 ml of methanol, filtered and used for HPLC analysis.

4.10. HPLC analyses

Instrumentation: Waters 600 system controller equipped with a Waters 490E multiwavelength detector and a Waters 717 plus Autosampler controlled by Millenium32 version 3.05 software; oven temperature: 40 °C; column: Zorbax Rx-C18, 4.6×150 mm, particle size 3.5 μm; guard column: Phenomenex C18, 4×3.00 mm; detection wavelength: 205 nm; injection volume: 10 μl; mobile phase A: 0.01% trifluoroacetic acid in water; mobile phase B: CH_3CN; flow rate: 1.00 ml/min; linear gradient: 0 min 80% A, 20% B; 5 min 80% A, 20% B; 10 min 50% A, 50% B; 31 min 46.5% A, 53.5% B; 55 min 5% A, 95% B; stop time: 60 min; post time: 15 min; Retention times (min): **1** 49.6, **2** 50.7, **3** 26.1, **4** 42.9, **5** 29.2, **6** 27.4, **7** 42.1, **8** 47.4, **9** 28.7, **10** 16.3, **11** 21.8, *N*-phenyl-undecanamide (internal standard) 44.1. All analyses were performed in triplicate. Amounts of compounds **1**–**11** were estimated by ratio of peak areas to areas of *N*-phenylundecanamide as internal standard (both measured at 205 nm).

4.11. LC–MS analyses

LC–MS analyses employing the HPLC system described above yielded only poor ionization of the analyzed compounds. Therefore, acetonitrile was replaced by methanol (+0.15% acetic acid) as the organic mobile phase (B) and 0.01% trifluoracetic acid in water by 0.15% acetic acid in water as the polar phase (A). Instrumentation: Hewlett Packard HP-1050 Liquid Chromatograph employed with a DAD-detector coupled with a Finnigan MAT SSQ 7000 mass spectrometer; oven temperature: 40 °C; column: Zorbax Rx-C18, 4.6×150 mm, particle size 3.5 μm; guard column: LiChroCart 4×4 mm packed with LiChrospher RP18 material (5 μm particle size); detection wave length: 205 nm; injection volume: 10 μl; mobile phase A: 0.15% acetic acid in water; mobile phase B: 0.15% acetic acid in MeOH; flow rate: 1.00 ml/min; linear gradient: 0 min 70% A, 30% B; 5 min 70% A, 30% B; 10 min 40% A, 60% B; 31 min 37.5% A, 63.5% B; 55 min 2% A, 98% B; stop time: 60 min; post time: 15 min; Retention times (min): **1** 47.4, **2** 46.9, **3** 40.3, **4** 45.3, **5** 40.0, **6** 40.8, **7** 44.9, **8** 43.1, **9** 34.1, **10** 19.2, **11** 31.0, *N*-phenyl-undecanamide (internal standard) 40.5.

MS parameters: APCI in the positive mode, employing a CID value of −5 V, a corona amperage of 5 μA, a sheath gas pressure of 50 psi, a capillary temperature of 150 °C, and a vaporizer temperature of 400 °C.

On-line MS fragmentation patterns observed for pure compounds **1**–**11** (relative signal-intensities are indicated in brackets): **1**: *m/z* 369 (8) $[M+2CH_3OH+H]^+$, 337 (72) $[M+CH_3OH+H]^+$, 319 (28) $[M-H_2O+CH_3OH+H]^+$, 305 (100) $[M+H]^+$, 287 (24) $[M-H_2O+H]^+$; **2**: *m/z* 291 (44) $[M+H]^+$, 273 (100) $[M-H_2O+H]^+$; **3**: *m/z* 367 (16) $[M-H_2O+2CH_3OH+H]^+$, 353 (24) $[M+CH_3OH+H]^+$, 335 (76) $[M-H_2O+CH_3OH+-H]^+$, 321 (8) $[M+H]^+$, 317 (36) $[M-2H_2O+CH_3OH+H]^+$, 303 (100) $[M-H_2O+H]^+$; **4**: *m/z* 395 (48) $[M+CH_3OH+H]^+$, 377 (24) $[M-H_2O+CH_3OH+H]^+$, 363 (64) $[M+H]^+$, 335 (40) $[M-CH_3COOH+CH_3OH+H]^+$, 317 (100) $[M-CH_3COOH\text{-}H_2O+CH_3OH+H]^+$, 303 (44) $[M-CH_3COOH+H]^+$, 285 (20) $[M-CH_3COOH\text{-}H_2O+H]^+$; **5**: *m/z* 307 (6) $[M+H]^+$, 289 (100) $[M-H_2O+H]^+$, 271 (32) $[M-2H_2O+H]^+$; **6**: *m/z* 289 (100) $[M-H_2O+H]^+$, 271 (28) $[M-2H_2O+H]^+$; **7**: *m/z* 321 (4) $[M+H]^+$, 289 (100) $[M-CH_3OH+H]^+$, 271 (26) $[M-CH_3OH\text{-}H_2O+H]^+$; **8**: *m/z* 291 (4) $[M-H_2O+2CH_3OH+H]^+$, 277 (8) $[M+CH_3OH+H]^+$, 259 (100) $[M-H_2O+CH_3OH+H]^+$, 245 (20) $[M+H]^+$, 227 (60) $[M-H_2O+H]^+$; **9**: *m/z* 289 (32) $[M-2H_2O+2CH_3OH+H]^+$, 275 (16) $[M-H_2O+CH_3OH+H]^+$, 257 (100) $[M-2H_2O+CH_3OH+H]^+$, 243 (16) $[M-H_2O+H]^+$, 225 (10) $[M-2H_2O+H]^+$. **10**: *m/z* 319 (36) $[M-2H_2O+2CH_3OH+H]^+$, 305 (12) $[M-H_2O+CH_3OH+H]^+$, 287 (100) $[M-2H_2O+CH_3OH+H]^+$, 273 (24) $[M-H_2O+H]^+$, 255 (50) $[M-2H_2O+H]^+$; **11**: *m/z* 393 (8) $[M-2H_2O+3CH_3OH+H]^+$; 361 (42) $[M-2H_2O+2CH_3OH+H]^+$, 347 (10) $[M-H_2O+CH_3OH+H]^+$, 329 (100) $[M-2H_2O+CH_3OH+H]^+$, 315 (20) $[M-H_2O+H]^+$.

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Appendix

Table I
HPLC quantification data (in mg/g dried plant material)[a]

Taxon	Sample	**1**	s_x	**2**	s_x	**3**	s_x	**4**	s_x	**5**	s_x	**6**	s_x	**7**	s_x	**8**	s_x	**9**	s_x
Anisotome																			
A. acutifolia	BR-010131-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.06	0.08	3.86	0.19
A. antipoda	CZ-000811-3	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	1.77	0.04
A. aromatica	CZ-000119-1	4.20	0.07	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-000121-8	2.08	0.01	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-000122-1	6.21	0.18	1.31	0.04	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.17	0.01
	CZ-000202-1	7.85	0.42	0.84	0.01	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	1.47	0.03
	CZ-000203-6	1.46	0.04	0.56	0.02	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	tr.	–	1.07	0.05
	CZ-000203-7	11.18	0.87	3.44	0.01	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.26	0.05
	CZ-000204-7	10.86	1.14	5.62	0.06	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.13	0.02
	CZ-000205-2	0.14	0.01	1.09	0.02	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.89	0.00
	CZ-010107B-1	2.32	0.09	7.38	0.20	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.20	0.01
	CZ-010225B-1	10.48	0.50	0.64	0.04	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.39	0.02
	CZ-010226A-1	1.04	0.03	0.48	0.05	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.69	0.01	0.11	0.01
A. brevistylis	CZ-001114-5	3.05	0.01	n.d.	–	n.d.	–	tr.	–	n.d.	–	n.d.	–	n.d.	–	0.74	0.04	0.55	0.02
	CZ-010126B-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.25	0.00	0.22	0.00
A. capillifolia	CZ-000128-4	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	1.79	0.02
A. cauticola	CZ-001115-4	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.97	0.00
A. deltoidea	CZ-010225C-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	tr.	–
	CZ-010226B-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
A. filifolia	CZ-000203-5	Tr.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	4.95	0.03	0.40	0.00
A. flexuosa	CZ-000121-7	14.09	0.92	2.45	0.01	n.d.	–	9.86	0.15	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-000122-2	13.00	0.91	1.25	0.01	n.d.	–	11.28	0.36	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-000123-1	13.94	0.96	1.52	0.03	n.d.	–	6.95	0.04	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.05	0.01
	CZ-000123-5	12.66	0.78	1.06	0.02	n.d.	–	10.39	0.21	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-000129-1	12.12	0.93	2.87	0.01	n.d.	–	n.d.	–	n.d.	–	0.19	0.00	n.d.	–	n.d.	–	1.20	0.01
	CZ-000205-1	5.56	0.14	1.16	0.00	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-010125E-1	5.34	0.12	0.41	0.02	n.d.	–	7.96	0.20	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.04	0.01
A. haastii	CZ-000127-1	0.14	0.01	10.73	0.65	n.d.	–	n.d.	–	3.66	0.01	1.66	0.02	0.49	0.03	n.d.	–	0.85	0.02
	CZ-000204-8	4.22	0.35	1.84	0.06	n.d.	–	n.d.	–	tr.	–	tr.	–	n.d.	–	n.d.	–	n.d.	–
A. imbricata var.	CZ-000123-4	8.49	0.59	7.79	0.32	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
imbricata	CZ-001114-3	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.30	0.01
A. imbricata var.	CZ-000204-6	10.92	0.61	1.99	0.01	n.d.	–	n.d.	–	–	–	n.d.	–	n.d.	–	0.77	0.03	0.99	0.01
prostrata	CZ-010125D-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.84	0.01
	SL-010225-15	4.29	0.07	0.30	0.03	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.27	0.02
A. lanuginosa	CZ-000121-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
	CZ-000121-4	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–
A. latifolia	CZ-000811-2	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	3.14	0.01	8.04	0.01
A. lyallii	CZ-000118-3	5.22	0.02	2.39	0.01	n.d.	–	n.d.	–	tr.	–	tr.	–	n.d.	–	n.d.	–	n.d.	–

(*continued on next page*)

Table I (*continued*)

Taxon	Sample	**1**	s_x	**2**	s_x	**3**	s_x	**4**	s_x	**5**	s_x	**6**	s_x	**7**	s_x	**8**	s_x	**9**	s_x
Anisotome																			
A. acutifolia	BR-010131-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.06	0.08	3.86	0.19
A. antipoda	CZ-000811-3	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	1.77	0.04
A. pilifera	CZ-000128-3	3.14	0.01	1.26	0.01	n.d.	–	n.d.	–	tr.	–	tr.	–	n.d.	–	n.d.	–	3.05	0.02
	CZ-000204-5	3.32	0.06	1.22	0.01	n.d.	–	n.d.	–	tr.	–	tr.	–	n.d.	–	n.d.	–	4.53	0.10
A. procumbens	GJ-001109-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	tr.	–
Aciphylla																			
Ac. aurea	CZ-000119-4	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	3.17	0.55
	CZ-001114-7	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	6.20	0.11
Ac. dieffenbachii	CZ-001123-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.33	0.00	7.16	0.11
Ac. scott-thomsonii	CZ-000811-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.28	0.06
Ac. similis	CZ-000204-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	tr.	–	16.52	1.43
Ac. subflabellata	CZ-001123-2	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.36	0.01	6.66	0.12
Apium																			
Ap. prostratum subsp. *prostr. var. filiforme*	CZ-001203-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.92	0.02	0.57	0.00
Gingidia																			
G. decipiens	CZ-001114-16	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	6.94	0.02	0.98	0.00
	CZ-010124C-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.46	0.01	4.28	0.00
G. montana	CZ-000127-2	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.45	0.01	5.85	0.28
Lignocarpa																			
L. carnosula[b]	CZ-010225-20	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	2.49	0.00
Oreomyrrhis																			
O. colensoi	CZ-000204-4	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.18	0.00	0.79	0.01
Scandia																			
S. geniculata	CZ-010323A-1	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.53	0.03	2.65	0.14

[a] Results of HPLC/UV investigations; amounts estimated by ratio of peak areas of *N*-phenylundecanamide as internal standard. All analyses were performed in triplicate; n.d.: not detectable; tr.; traces, i.e. <0.10 mg/g.

[b] Estimated contents of **10** and **11**: 5.95 ± 0.46 mg/g and 4.91 ± 0.08 mg/g, respectively.

Table II
Origin of plant material[a]

Taxon	Location[b]	Co-ordinates	Altitude (a.m.s.l.)	Sample (date-nr.)
Anisotome				
A. acutifolia[c]	NZ/SL/North-east Island, Snares Islands	S 48° 02′; E 166° 36′	40 m	BR-010131-1
A. antipoda[d]	NZ/Adams Island, Auckland Islands	S 50° 50′; E 165° 55′	20 m	CZ-000811-3
A. aromatica	NZ/OT/Mount Maungatua	S 45° 52′; E 170° 07′	835 m	CZ-000119-1
	NZ/OT/Old Man Range	S 45° 21′; E 169° 12′	1460 m	CZ-000121-8
	NZ/OT/Mount Moha, Dunstan Mountains	S 44° 59′; E 169° 26′	1220 m	CZ-000122-1
	NZ/SL/Falls Creek	S 44° 50′; E 168° 03′	870 m	CZ-000129-1
	NZ/CB/S Castle Hill near Castle Hill Village	S 43° 14′; E 171° 43′	780 m	CZ-000202-1
	NZ/CB/Ribbonwood Stream, Arthur's Pass	S 43° 05′; E 171° 45′	1100 m	CZ-000203-6
	NZ/CB/Ribbonwood Stream, Arthur's Pass	S 43° 05′; E 171° 45′	1100 m	CZ-000203-7
	NZ/CB/Temple Basin ski area, Arthur's Pass	S 42° 54′; E 171° 35′	1425 m	CZ-000204-7
	NZ/CB/Broken River ski area, Craigieburn Range	S 43° 07′; E 171° 41′	1550 m	CZ-000205-1
	NZ/CB/Broken River ski area	S 43° 08′; E 171° 41′	1250 m	CZ-000205-2
	NZ/OT/Rock and Pillar Range	S 45° 34′; E 169° 55′	830 m	CZ-010107B-1
	NZ/NE/Mount Arthur	S 41° 13′; E 172° 42′	1530 m	CZ-010225B-1
	NZ/NE/Lake Sylvester	S 41° 06′; E 172° 38′	1350 m	CZ-010226A-1

(*continued on next page*)

Table II (*continued*)

Taxon	Location[b]	Co-ordinates	Altitude (a.m.s.l.)	Sample (date-nr.)
A. brevistylis	NZ/OT/Dunstan Mountains	S 45° 02′; E 169° 18′	1080 m	CZ-001114-5
	NZ/OT/between Lake Hawea and Lawyer Burn Hut	S 44° 26′; E 169° 16′	670 m	CZ-010126B-1
A. capillifolia	NZ/SL/Falls Creek	S 44° 49′; E 168° 01′	1080 m	CZ-000128-4
A. cauticola	NZ/OT/Nevis Valley	S 45° 09′; E 169° 07′	960 m	CZ-001115-4
A. deltoidea	NZ/NE/Mount Arthur	S 41° 12′; E 172° 42′	1520 m	CZ-010225C-1
	NZ/NE/Lake Sylvester	S 41° 06′; E 172° 38′	1360 m	CZ-010226B-1
A. filifolia	NZ/CB/Ribbonwood Stream, Arthur's Pass	S 43° 05′; E 171° 45′	920 m	CZ-000203-5
A. flexuosa	NZ/OT/Old Man Range	S 45° 21′; E 169° 12′	1480 m	CZ-000121-7
	NZ/OT/Dunstan Mountains	S 44° 59′; E 169° 26′	1210 m	CZ-000122-2
	NZ/OT/Crown Range	S 44° 59′; E 168° 57′	870 m	CZ-000123-1
	NZ/OT/Pisa Range	S 44° 52′; E 169° 07′	1420 m	CZ-000123-5
	NZ/OT/Pisa Range	S 44° 54′; E 169° 12′	1420 m	CZ-010125E-1
A. haastii	NZ/SL/Falls Creek	S 44° 50′; E 168° 04′	1080 m	CZ-000127-1
	NZ/CB/Temple Basin ski area, Arthur's Pass	S 42° 54′; E 171° 35′	1425 m	CZ-000204-8
A. imbricata var. *imbr.*	NZ/OT/Pisa Range	S 44° 52′; E 169° 07′	1540 m	CZ-000123-4
	NZ/OT/Northburn Station, Dunstan Mountains	S 45° 02′; E 169° 21′	1560 m	CZ-001114-3
A. imbricata var. *prostrata*	NZ/CB/Temple Basin ski area, Arthur's Pass	S 42° 54′; E 171° 35′	1425 m	CZ-000204-6
	NZ/OT/Pisa Range	S 44° 53′; E 169° 11′	1830 m	CZ-010125D-1
	NZ/MB/Mt. Terako, Amuri Range	S 42° 28′; E 173° 12′	1700 m	SL-010225-15
A. lanuginosa	NZ/OT/Old Man Range	S 45° 23′; E 169° 13′	1700 m	CZ-000121-1
	NZ/OT/Old Man Range	S 45° 21′; E 169° 12′	1640 m	CZ-000121-4
A. latifolia[d]	NZ/Adams Island, Auckland Islands	S 50° 50′; E 165° 55′	20 m	CZ-000811-2
A. lyallii	NZ/OT/Cannibal Bay	S 46° 28′, E 169° 46′	10 m	CZ-000118-3
A. pilifera	NZ/SL/Falls Creek	S 44° 50′; E 168° 02′	1070 m	CZ-000128-3
	NZ/CB/Temple Basin ski area, Arthur's Pass	S 42° 54′; E 171° 35′	1425 m	CZ-000204-5
A. procumbens[e]	AU/TA/Mt. Anne, Tasmania	S 42° 57′; E 146° 25′	1400 m	GJ-001109-1
Aciphylla				
Ac. aurea	NZ/OT/Mount Maungatua	S 45° 52′; E 170° 07′	710 m	CZ-000119-4
	NZ/OT/Dunstan Mountains	S 45° 02′; E 169° 18′	840 m	CZ-001114-7
Ac. dieffenbachii[f]	NZ/Chatham Islands	n.a.	n.a.	CZ-001123-1
Ac. scott-thomsonii	NZ/OT/Swampy Hill near Dunedin	S 45° 47′; E 170° 29′	680 m	CZ-000811-1
Ac. similis	NZ/CB/Temple Basin ski area, Arthur's Pass	S 42° 55′; E 171° 34′	1130 m	CZ-000204-1
Ac. subflabellata[f]	NZ/CB/Porter's Pass	n.a.	n.a.	CZ-001123-2
Apium				
Ap. prostratum subsp. *prostratum* var. *filiforme*	NZ/OT/Blackhead beach E Waldronville	S 45° 56′, E 170° 26′	10 m	CZ-001203-1
Gingidia				
G. decipiens	NZ/OT/Dunstan Mountains	S 45° 01′; E 169° 17′	730 m	CZ-001114-6
	NZ/OT/Shotover Valley near Queenstown	S 44° 51′; E 168° 41′	510 m	CZ-010124C-1
G. montana	NZ/SL/Falls Creek	S 44° 50′; E 168° 04′	930 m	CZ-000127-2
Lignocarpa				
L. carnosula	NZ/MB/Mt. Terako, Amuri Range	S 42° 27′; E 173° 11′	1600 m	SL-010225-20
Oreomyrrhis				
O. colensoi	NZ/CB/Temple Basin ski area, Arthur's Pass	S 42° 55′; E 171° 35′	1400 m	CZ-000204-4
Scandia				
S. geniculata	NZ/OT/Heyward Point	S 45° 46′; E 170° 42′	130 m	CZ-010323A-1

[a] Plants were collected by CZ in the wild unless stated otherwise.

[b] Abbreviations of country names and political districts: AU: Australia, NZ: New Zealand; CB: Canterbury, MB: Marlborough, NE: Nelson, OT: Otago, SL: Southland, TA: Tasmania.

[c] Sample of a cultivated plant grown at Invercargill made available by B. Rance.

[d] Samples of cultivated plants grown at Invermay made available by A. Evans.

[e] Collected in the wild by G. Jordan.

[f] Samples from plants cultivated in the Dunedin Botanical Garden made available by T. Myers.

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