

# Composition of the Essential Oils of Blackcurrant Buds (*Ribes nigrum* L.)

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The essential oils of 23 varieties of blackcurrant buds were investigated for chemical composition and chemosystematic studies. They were fractionated into hydrocarbons and oxygenated compounds. The two fractions were submitted to gas chromatography (GC), GC/mass spectrometry, and GC/Fourier transform infrared spectrometry. A total of 86 compounds were detected in significant amount, 26 of which for the first time as blackcurrant constituents. Among them, 11 were definitely characterized. Five sesquiterpene hydrocarbons:  $\delta$ -elemene,  $\gamma$ -muurolene, ledene, bicyclogermacrene, germacrene B. Six oxygenated compounds: *p*-menth-2-en-1-ol (trans and cis isomers), *cis*-limonene 1,2-epoxide, linalyl and neryl acetates, isospathulenol. Their importance is discussed in terms of concentration and flavor impact.

Blackcurrant (*Ribes nigrum* L.) is a shrub spontaneously present throughout the cold and temperate zones. It is grown extensively in Asia (USSR, Tibet), Australia (Tasmania), and Europe for its berries traditionally used for the preparation of fruit pulp or juice and in alcoholic beverages.

Absolutes and oils obtained from dormant buds are now used as flavoring or flavor enhancer in cosmetics and food products. The essential oil of blackcurrant buds gives off a strong terpenic flavor overwhelmed by a "catty" note.

Early work on the composition of this essential oil was reviewed by Williams (1972). Further studies on buds oil have shown that the essential oil composition is a discriminating feature, characteristic of each cultivar (Latrasse and Lantin, 1974, 1977). Chemotypes, based on the apolar fraction of the oil, were defined in terms of major monoterpene and sesquiterpene contents (Latrasse, 1983). Recent studies on the chemical composition of buds oil, based on a limited number of varieties, left many compounds unidentified (Derbesy et al., 1980; Kerslake and Menary, 1985), among which are major compounds, especially sesquiterpene hydrocarbons and oxides, and some minor components of the oxygenated fraction, which are important flavor impact compounds. Two important constituents were recently identified in this fraction, i.e. the sesquiterpene alcohol (+)-spathulenol (Le Quere and Latrasse, 1986) and the sulfur-containing compound 4-methoxy-2-methyl-2-mercaptobutane, responsible for the characteristic catty note of blackcurrant (Rigaud et al., 1986).

The present study was undertaken in order to increase the knowledge of the chemical composition and of the chemotaxonomy of the essential oils of blackcurrant buds. Attention has been particularly paid on the identification of two sesquiterpene hydrocarbons that were shown to be discriminative in a recent chemotaxonomic study (Kerslake et al., 1989) and on the composition of the oxygenated fraction. Olfactory properties of some major compounds in this fraction have been also underlined. Gas chromatography (GC), mass spectrometry in electron impact and chemical ionization modes (GC/MS), and Fourier transform infrared spectrometry (GC/FTIR) have been used to analyze the essential oils of blackcurrant buds of 23 varieties.

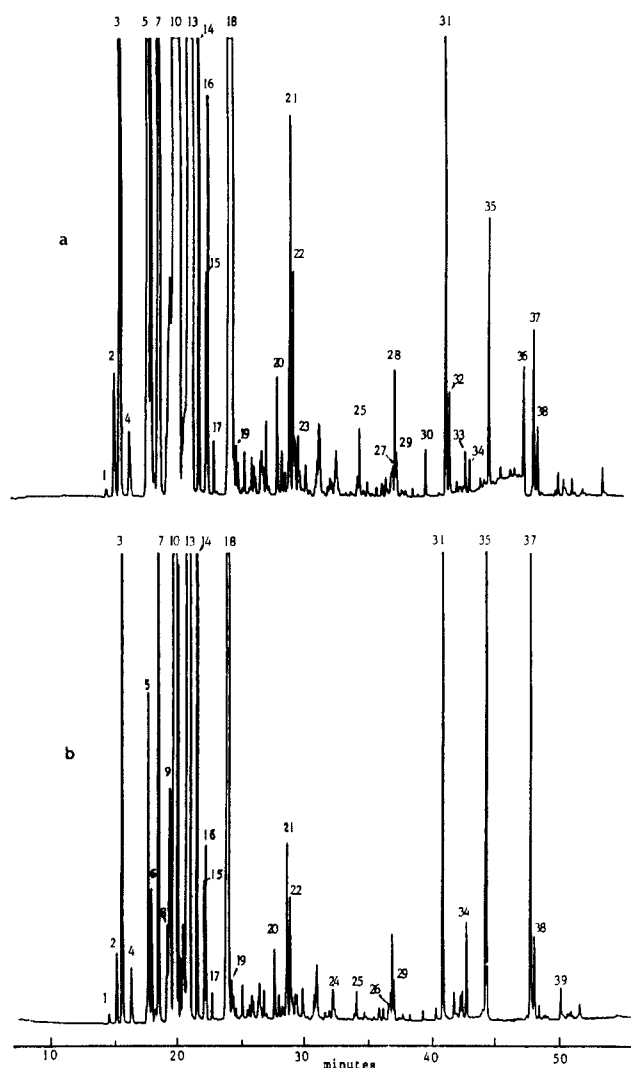
## EXPERIMENTAL SECTION

**Recovery of the Essential Oils.** Dormant buds of 23 varieties of diverse origins and ancestries were collected during January 1987 in different research centers (Station de Recherches d'Arboriculture Fruitière in Angers and Laboratoire des Petits Fruits in Dijon) of the Institut National de la Recherche Agronomique (INRA) and stored at  $-18^{\circ}\text{C}$  before use. [These 23 varieties were Golubka, Cotswold Cross, Malvern Cross, Tor Cross, Baldwin, White Bud, Magnus, Wellington XXX, Hatton Black, Silvergieter, Boskoop Giant, Kerry, Noir de Bourgogne, Burga, Tchernaja L., Davidson's 8, Tifon, Tenah 4, Royal de Naples, Brødrtorp, Troll, Öjebyn, and Andega.] Approximately 7 g of buds frozen into liquid  $\text{N}_2$  was ground in a stainless steel blender. A 5-g portion of the powder was suspended in 1300 mL of purified water and steam-distilled in a Likens-Nickerson device for 30 min with pentane as a solvent. The organic extracts were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated at  $40^{\circ}\text{C}$  under atmospheric pressure in a microdistillation apparatus. Ultimate concentrations were carried out with a micro Dufton column, made and experimented for potential loss of volatiles as described by Guichard and Ducruet (1984). A concentrate of ca. 0.5 mL for each varietal sample was submitted to fractionation.

**Fractionation of the Concentrates.** The concentrates were fractionated in a jacketed column at  $15^{\circ}\text{C}$  on 3 g of Kieselgel 60 (5-20- $\mu\text{m}$  diameter, Merck) hydrated to 25% (w/w) prepared as previously described (Latrasse et al., 1982). The hydrocarbons were eluted with 50 mL of purified pentane, and the oxygenated fractions were eluted with 50 mL of distilled dichloromethane. The eluates were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated as mentioned above. The final concentrations corresponded to ca. 500  $\mu\text{L/L}$ .

**Gas Chromatography.** For GC separations and quantifications, a 1- $\mu\text{L}$  sample was injected (splitless) onto a fused silica capillary column [length 30 m, i.d. 0.32 mm; bonded stationary-phase DB5 (J&W Scientific); thickness 1  $\mu\text{m}$ ] and chromatographed under the following conditions: carrier gas (helium) velocity, 31 cm/s; temperature program, from 45 to  $220^{\circ}\text{C}$  at  $3^{\circ}\text{C}/\text{min}$ ; injection port and detector (FID) temperature,  $240^{\circ}\text{C}$ . Quantitation of peaks was expressed as a percentage of the total peak area. Measurements of peak areas were achieved by using an electronic integrator (Enica 10, Delsi Instruments).

**Gas Chromatography/Mass Spectrometry.** The GC/MS analyses were performed with a Nermag R 10-10 C mass spectrometer coupled to a Girdel 31 gas chromatograph. Bonded-phase fused silica capillary columns, DB5 (60 m  $\times$  0.32 mm (i.d.), 1- $\mu\text{m}$  film thickness, J&W Scientific) and DBWax (30 m  $\times$  0.32 mm (i.d.), 0.5- $\mu\text{m}$  film thickness, J&W Scientific) were used for GC/MS determinations. The carrier gas (He) velocity was 35 cm/s, and the column was directly connected to the



**Figure 1.** Gas chromatograms of the bud essential oils of two varieties of blackcurrant: Royal de Naples (a) and Noir de Bourgogne (b). Conditions: column DB5 (30 m × 0.32 mm (i.d.), 1- $\mu$ m phase thickness); column temperature, 45–220 °C at 3 °C/min; carrier gas, helium; velocity, 31 cm/s. For the identity of the numbered peaks, refer to Table I.

ion source of the mass spectrometer. The split/splitless injection port was maintained at 240 °C, and oven temperature was programmed from 40 to 220 °C at 3 °C/min. Kovats indices were calculated using *n*-alkanes C<sub>7</sub>–C<sub>22</sub> as external references (Van den Dool and Kratz, 1963).

Electron impact mass spectra were recorded at 70 eV with a source temperature of 150 °C. Chemical ionization spectra, using either methane or ammonia with a source pressure of 0.3 Torr, were obtained at 90 eV with a source temperature of 90 °C.

**Gas Chromatography/Fourier Transform Infrared Spectrometry.** The gas-phase infrared spectra were obtained on a Bruker IFS 85 Fourier transform spectrometer, connected via a gold-coated light pipe (20 cm × 0.8 mm (i.d.)) to a Carlo-Erba 5160 gas chromatograph equipped with an on-column injector and a flame ionization detector. The same capillary columns as described above were used with a helium flow rate of about 2 mL/min (velocity 35 cm/s). Oven temperature was programmed from 40 to 200 °C at 3 °C/min.

Nitrogen makeup gas was introduced into the transfer line to reduce peak broadening within the light pipe (maintained at 200 °C), resulting in a total gas flow rate of 5 mL/min.

The spectral resolution was 8 cm<sup>-1</sup>, and time resolution was reduced from 12 collected interferograms/s to 4 effective ones by coadding 3 interferograms in real time. A narrow-band (4800–600 cm<sup>-1</sup>) mercury-cadmium-telluride (MCT) detector was used.

**Reference Compounds.** Commercial chemicals were used as references when available.  $\delta$ -Elemene,  $\beta$ -elemene,  $\gamma$ -

**Table I.** Major Constituents of the Essential Oils of Blackcurrant Buds and Their Percentage Composition<sup>a</sup>

peak no. <sup>b</sup>	compound	rel peak area, <sup>c</sup> %
2	$\alpha$ -thujene	tr-1.90
3	$\alpha$ -pinene	1.30-9.23
4	camphene	tr-2.41
5	sabinene	tr-69.00
6	$\beta$ -pinene	0.17-10.22
7	myrcene	2.89-3.61
8	4-carene	tr-0.55
9	$\alpha$ -phellandrene	tr-1.58
10	$\Delta^3$ -carene	0.17-45.50
	$\alpha$ -terpinene	tr-1.32
	<i>m</i> -cymene	tr-0.17
	<i>p</i> -cymene	tr-0.48
13	limonene	0.17-14.12
13	$\beta$ -phellandrene	1.72-24.63
	( <i>Z</i> )-ocimene	0.00-5.63
	( <i>E</i> )-ocimene	1.21-3.12
15	unknown 1	tr-0.32
16	$\gamma$ -terpinene	tr-1.80
17	<i>trans</i> -sabinene hydrate	tr-0.54
18	terpinolene	0.40-20.00
19	<i>cis</i> -sabinene hydrate	0.00-0.46
	<i>trans</i> - <i>p</i> -menth-2-en-1-ol	0.00-0.18
	limonene 1,2-epoxide	0.00-0.20
	<i>cis</i> - <i>p</i> -menth-2-en-1-ol	0.00-0.18
	<i>p</i> -mentha-1,5-dien-8-ol	tr-0.66
21	terpinen-4-ol	0.40-4.52
22	<i>p</i> -cymen-8-ol	tr-0.66
23	$\alpha$ -terpineol	tr-0.22
	citronellol	0.00-0.18
24	linalyl acetate	tr-0.12
25	bornyl acetate	0.00-2.00
26	unknown 9	0.00-0.18
27	$\delta$ -elemene	tr-0.24
28	citronellyl acetate	tr-0.42
29	$\alpha$ -terpinyl acetate	tr-0.40
30	$\beta$ -elemene	tr-0.14
31	$\beta$ -caryophyllene	1.20-4.98
32	$\gamma$ -elemene	0.00-1.62
33	$\alpha$ -humulene	tr-2.00
34	<i>allo</i> -aromadendrene	tr-0.33
	germacrene D	0.00-1.51
35	bicyclogermacrene	tr-3.54
	$\delta$ -cadinene	tr-0.15
36	germacrene B	0.00-1.12
37	spathulenol	tr-2.70
38	caryophyllene oxide	tr-1.48
	humulene epoxide	0.00-0.52
	unknown 10	0.00-0.59
39	isopathulenol	0.00-0.18
	unknown 11	0.00-0.12

<sup>a</sup> Percentages were calculated from the peak area of the unfractionated essential oils. FID response factors were not determined. <sup>b</sup> Peak numbers according to Figure 1. <sup>c</sup> Extreme values obtained for 23 varieties. tr = less than 0.1%.

elemene, and germacrene B were a present from F. Van den Berg, TNO-CIVO, Zeist, the Netherlands. Isopathulenol was a gift from Dr. Maurer (Firmenich, Switzerland). Germacrene D was obtained from an essential oil of caraway as a gift of Dr. Kerslake, University of Tasmania, Hobart, Australia. Sabinene hydrates were a present from Dr. Fischer, Technische Universität München, FRG.

## RESULTS AND DISCUSSION

Steam distillation of the buds, previously frozen into liquid nitrogen and reduced in powder, allows recovery of from 2 to 5 mL of essential oil/kg of material. The gas chromatograms in Figure 1 show the compositions of the volatile buds oils of two varieties of blackcurrant. They were chosen from among the 23 investigated varieties for illustration, owing to their different but characteristic hydrocarbon chemotypes (Kerslake et al., 1989).

**Table II. Hydrocarbons Identified in the Pentane Fraction of the Essential Oils of Blackcurrant Buds**

compound	MW (CI/MS)	Kovats ret index (KI)		reliability of identification <sup>a</sup>
		DB5 (GC/MS)	DB Wax (GC/MS)	
Monoterpenes				
$\alpha$ -thujene	136	923	1021	b
$\alpha$ -pinene	136	932	1015	a
camphene	136	946		b
sabinene	136	972	1118	a
$\beta$ -pinene	136	978	1103	a
myrcene	136	987	1164	a
4-carene	136	1001	1128	d
$\alpha$ -phellandrene	136	1005	1167	b
$\Delta^3$ -carene	136	1014	1149	a
$\alpha$ -terpinene	136	1019	1177	b
<i>m</i> -cymene	134	1023	1267	c
<i>p</i> -cymene	134	1026	1269	a
limonene	136	1029	1196	a
$\beta$ -phellandrene	136	1034	1207	b
( <i>Z</i> )-ocimene	136	1037	1237	c
( <i>E</i> )-ocimene	136	1048	1253	b
unknown 1	136	1059		
$\gamma$ -terpinene	136	1062		a
terpinolene	136	1092	1284	a
<i>p</i> -mentha-1,3,8-triene <sup>c</sup>	134	1114	1391	d
<i>allo</i> -ocimene <sup>c</sup>	136	1129	1372	c
Sesquiterpenes				
$\delta$ -elemene <sup>c</sup>	204	1347	1464	a
$\alpha$ -copaene	204	1392		a
$\beta$ -elemene	204	1406		a
$\beta$ -guaiene <sup>c</sup>	204	1423		c
$\beta$ -caryophyllene	204	1440	1591	a
$\gamma$ -elemene	204	1445	1633	a
$\beta$ -cubebene <sup>c</sup>	204	1458		c
aromadendrene	204	1460	1605	a
$\alpha$ -gurjunene <sup>c</sup>	204	1468		d
$\alpha$ -humulene	204	1473	1665	a
<i>allo</i> -aromadendrene	204	1483	1643	a
$\gamma$ -muurolene <sup>c</sup>	204	1486	1684	b
germacrene D	204	1500	1702	a
bicyclogermacrene <sup>c</sup>	204	1515	1728	b
ledene <sup>c</sup>	204	1518		a
$\gamma$ -cadinene	204	1536		b
$\delta$ -cadinene	204	1538	1753	b
viridiflorene <sup>c</sup>	204	1548		d <sup>b</sup>
germacrene B <sup>c</sup>	204	1582	1823	a

<sup>a</sup> Key for reliability of identification: a = MS, KI, and IR identical with those of pure reference compounds; b = MS and KI identical with published data, consistent with CI-MS and IR; c = MS and KI identical with published data, consistent with CI-MS; d = MS identical with published spectra. <sup>b</sup> Swords and Hunter, 1978. <sup>c</sup> Newly identified in blackcurrant buds essential oils.

Peak identities of major components and a range of variation of their relative amounts in the 23 oils are given in Table I. Only those compounds with a relative amount above 0.1% in at least one oil were included (50 compounds).

For easier identification of individual components, the oils were fractionated into hydrocarbons and oxygenated compounds. Identification of all the compounds was based on their mass spectra and further supported by comparison of their Kovats retention indices, measured on DB5 and DBWax columns, and of their infrared spectra with those of authentic samples. When those samples were not available, identifications were made with the aid of mass spectral data bases (EPA/NIH, 1978; ten Noever de Brauw et al., 1987; *Eight Peak Index of Mass Spectra*, 1974) and published retention indices and mass spectra (Jennings and Shibamoto, 1980; Nykanen, 1986a,b; Tressl et al., 1983; Okamoto et al., 1981; Koyasako and Bernhard, 1983). Gas-phase infrared spectra were also

**Table III. Oxygen-Containing Compounds Identified in the Methylene Chloride Fraction of the Essential Oils of Blackcurrant Buds**

compound	MW (CI/MS)	Kovats ret index (KI)		reliability of identification <sup>a</sup>
		DB5 (GC/MS)	DB Wax (GC/MS)	
4-methoxy-2-methyl-2-mercaptobutane	134	910		a
1-octen-3-ol	128		1455	a
1,8-cineole	154	1033	1206	a
<i>trans</i> -sabinene hydrate	154	1069	1468	a
linalool	154	1095	1553	a
<i>cis</i> -sabinene hydrate	154	1098	1553	a
<i>trans</i> - <i>p</i> -menth-2-en-1-ol <sup>d</sup>	154	1124	1630	b
<i>cis</i> -limonene 1,2-epoxide <sup>d</sup>	152	1139	1444	a
<i>cis</i> - <i>p</i> -menth-2-en-1-ol <sup>d</sup>	154	1145	1565	c
unknown 2	152	1147	1464	
<i>p</i> -mentha-1,5-dien-8-ol <sup>d</sup>	152	1165	1718	c <sup>b</sup>
<i>p</i> -mentha-1,3-dien-8-ol <sup>d</sup>	152	1172		d
unknown 3				
terpinen-4-ol	154	1182	1601	a
<i>m</i> -cymen-8-ol <sup>d</sup>	150		1845	c
<i>p</i> -cymen-8-ol	150	1186	1850	a
criptone <sup>d</sup>	138	1188		d
$\alpha$ -terpineol	154	1193	1697	a
<i>trans</i> -piperitol		1202	1679	d
unknown 4	152		1826	
<i>p</i> -menth-2-en-7-ol <sup>d</sup>			1778	d
<i>cis</i> -piperitol		1215	1747	d
citronellol	156		1771	a
umbellulone <sup>d</sup>	150		1667	d
linalyl acetate <sup>d</sup>	196	1252	1557	a
unknown 5	154	1254	1527	
unknown 6	150		1743	
bornyl acetate	196	1290	1577	a
terpinen-4-yl acetate	196	1304	1619	b
unknown 7	150			
unknown 8		1333	1670	
unknown 9	196		1672	
citronellyl acetate	198	1348	1662	a
$\alpha$ -terpinyl acetate	196	1353	1692	a
neryl acetate <sup>d</sup>	196	1363		a
geranyl acetate	196	1380	1756	a
spathulenol	220	1601	2121	a
caryophyllene oxide isomer	220		1966	d
caryophyllene oxide	220	1609	1975	a
humulene oxide	220		2007	d
unknown 10	220	1638	2032	
isopathulenol	220	1662	2250	
isomer <sup>d</sup>				
isopathulenol <sup>d</sup>	220	1666	2222	a <sup>c</sup>
$\alpha$ -cadinol <sup>d</sup>	222	1676	2227	b
unknown 11	220		2348	
6,10,14-trimethyl-2-pentadecanone <sup>d</sup>	268	1846		d

<sup>a</sup> See footnote a, Table I. <sup>b</sup> Lamparsky and Klimes, 1985. <sup>c</sup> Maurer and Hauser, 1983. <sup>d</sup> Newly identified in blackcurrant buds essential oils.

compared with published IR spectra (Herres, 1987; Herres et al., 1986). From the 86 compounds listed in Tables II and III, 75 were identified and most of them were found in significant amounts in all the cultivars (Table I). Mass spectral data of the remaining 11 unidentified products are reported briefly in Table IV.

Table IV. Mass Spectra (Eight Prominent Ions in Order of Decreasing Abundance) of the Unknown Compounds 1-11<sup>a</sup>

unknown compound	MW (CI/MS)	EI/mass spectra, <i>m/e</i> (rel intens, %)								suggested structure <sup>b</sup>
1	136	93(100)	91(41)	43(29)	77(28)	92(26)	41(17)	136(15)	79(15)	monoterpene
2	152	79(100)	110(68)	95(37)	41(30)	39(29)	43(26)	77(24)	93(20)	terpene alcohol
3		109(100)	43(23)	91(19)	41(17)	79(12)	77(12)	81(11)	110(11)	
4	152	43(100)	91(28)	109(27)	41(26)	81(22)	39(22)	119(18)	55(18)	terpene alcohol
5	154	93(100)	43(91)	41(33)	136(23)	92(19)	121(17)	55(12)	69(11)	
6	150	150(100)	41(97)	39(77)	107(75)	91(75)	79(52)	108(51)	135(41)	terpene ketone
7	150	150(100)	91(78)	107(54)	79(53)	39(52)	41(51)	77(42)	117(31)	terpene ketone
8		93(100)	43(56)	92(37)	91(19)	41(17)	136(15)	121(10)	79 (9)	
9	196	93(110)	43(92)	41(30)	79(24)	121(23)	136(20)	94(20)	67(18)	terpene acetate <sup>c</sup>
10	220	43(100)	41(62)	109(61)	138(52)	96(49)	67(48)	55(38)	53(24)	sesquiterpene oxide <sup>c</sup>
11	220	43(100)	41(56)	162(50)	119(44)	121(38)	105(32)	107(30)	220(28)	sesquiterpene alcohol <sup>c</sup>

<sup>a</sup> See Tables I and II. <sup>b</sup> On the basis of CI spectra and/or IR spectra. <sup>c</sup> Main absorptions of the FT/IR spectra: 9 = 1040, 1232, 1763, 2966 cm<sup>-1</sup>; 10 = 972, 1076, 1389, 1454, 2964 cm<sup>-1</sup>; 11 = 955, 1090, 1381, 1441, 2922, 3641 cm<sup>-1</sup>.

**Hydrocarbon Fraction.** The hydrocarbon fraction was the main part of the essential oil for all the cultivars, with a relative amount varying from 90 to 95% of the oils. Among this, monoterpenes constituted the major part (mean of 87%), the remaining being sesquiterpenes.

Composition of the major monoterpene hydrocarbons was found in good agreement with previous observations (Latrasse, 1983; Kerslake and Menary, 1985) and confirmed the monoterpene chemotypes previously described (Latrasse and Lantin, 1977). Major monoterpenes were  $\alpha$ - and  $\beta$ -pinenes, sabinene,  $\Delta^3$ -carene, limonene,  $\beta$ -phellandrene, and terpinolene. Combined in different ways depending on the cultivars, they represented from 66.0% to 75.5% of the total oils.

The hydrocarbons *m*-cymene and *p*-cymene had identical mass spectra, but on the basis of Kovats indices, the structure *m*-cymene was proposed for the earlier eluting isomer (Randriamiharisoa and Gaydou, 1987).

Identity of the major sesquiterpene hydrocarbons was in good agreement with our previous observations (Kerslake et al., 1989). However, sesquiterpenes previously described as unknowns I and II were identified as bicyclogermacrene and germacrene B, respectively. Other important sesquiterpenes were  $\delta$ -elemene,  $\beta$ -caryophyllene,  $\gamma$ -elemene,  $\alpha$ -humulene, alloaromadendrene, and germacrene D.

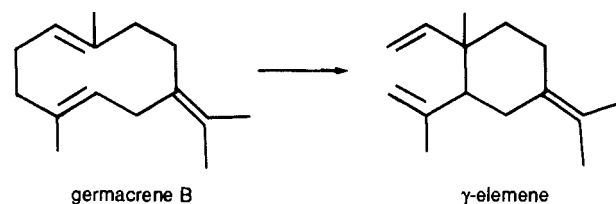
One sesquiterpene,  $\beta$ -caryophyllene, was the main sesquiterpene hydrocarbon in all but one of the studied cultivars (Noir de Bourgogne). Its amount ranged from 1.20% to 4.98% of the total oils. Converse to previous observations (Derbesy et al., 1980; Kerslake and Menary, 1985),  $\alpha$ -copaene was only found as traces. The difference could however reflect the different methods used for the isolation of the oils (steam distillation of the buds versus steam distillation of absolutes or vacuum distillation of concretes).

Three sesquiterpenes (Figure 1, peaks 32, 35, and 36), particularly important for the chemotypic description (Kerslake et al., 1989), had closely related mass spectra.

Spectral search in data bases gave essentially four candidates:  $\gamma$ -elemene, bicycloelemene, bicyclogermacrene, germacrene B. In all chromatograms in which the first peak (retention index 1445 on DB5) appeared, the last eluting one (retention index 1582 on DB5) also occurred. Chromatography on the apolar DB5 column of fractions containing both compounds gave broad peaks in the sesquiterpene region (Figure 1a). These broad peaks were completely absent in the chromatograms obtained on a polar column (DBWax). This suggested a rearrangement process occurring at high temperature during the chromatography, such as Cope rearrangement already signaled for cyclodeca-1,5-dienes when chromatographed on

apolar columns (Weinheimer et al., 1970; Burger et al., 1985; Herres et al., 1986; Clark et al., 1987). The IR spectrum of the first isomer (peak 32) displayed characteristic absorption bands for terminal methylene groups ( $\delta_{CH} = 895, 910 \text{ cm}^{-1}$ ). These absorptions were absent in the spectrum of peak 36. Finally, the former was positively identified as  $\gamma$ -elemene by injection of an authentic sample. The structure of the latter was therefore supposed to be germacrene B, and this was confirmed with the reference compound.

Injection of a pure sample of germacrene B onto a DB5 column through a hot injector resulted in important amounts of  $\gamma$ -elemene (formed by thermal isomerization in the hot injector) and a broad peak eluting from  $\gamma$ -elemene up to germacrene B, resulting from the following Cope rearrangement, which occurs during the chromatography, as already mentioned by Clark et al. (1987):



Since chromatography of germacrene B onto a DBWax column did not generate any broad peak, owing to the lower temperature of elution on this polar column, and, moreover, since cold on-column injections onto this polar column never allowed detection of  $\gamma$ -elemene, one can conclude that  $\gamma$ -elemene is an artifact in these analyses. Finally, gas-phase IR spectra of  $\gamma$ -elemene and germacrene B were identical with those published by Herres (1987).

The mass spectrum and gas-phase IR spectrum of the third compound (peak 35) are displayed in Figure 2. Its IR spectrum, where characteristic absorptions for terminal methylene groups were absent, allowed tentative identification as bicyclogermacrene. This was mostly confirmed by its Kovats index (1728 on DBWax) in good agreement with published retention index on Carbowax 20M (Malingré and Maarse, 1974).

Indirect evidence for this attribution of structure was also obtained when a new important peak, with a mass spectrum almost identical (Figure 3), appeared at a much lower retention time ( $I_K$ (DB5) 1345), when the injection port temperature was increased to 300 °C. Since this peak was never found with an injector below 240 °C, this again suggested a Cope rearrangement and the new peak was therefore tentatively identified as bicycloelemene (Nishimura et al., 1969). Bicyclogermacrene is the leading sesquiterpene of the variety Noir de Bourgogne, where

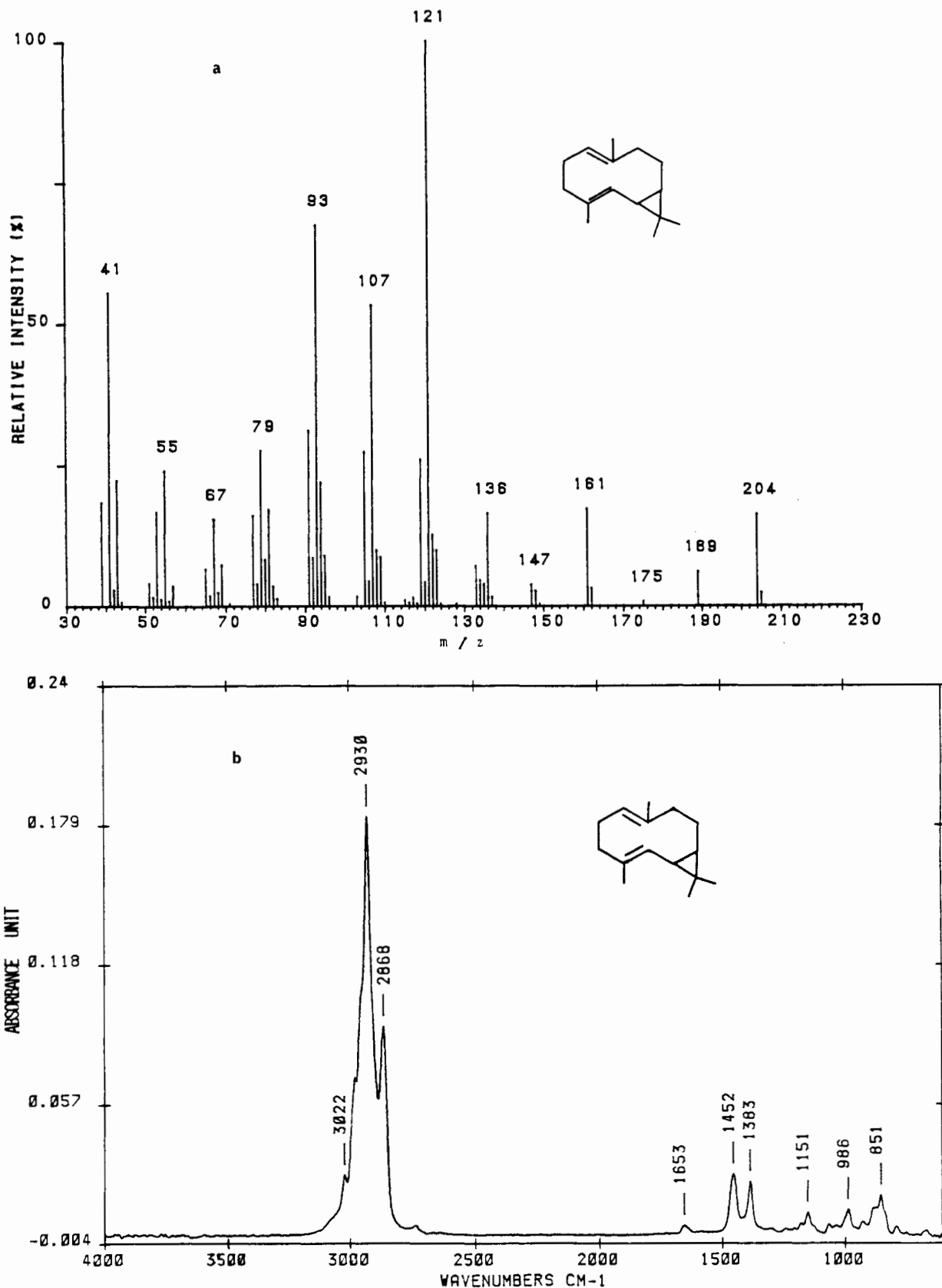


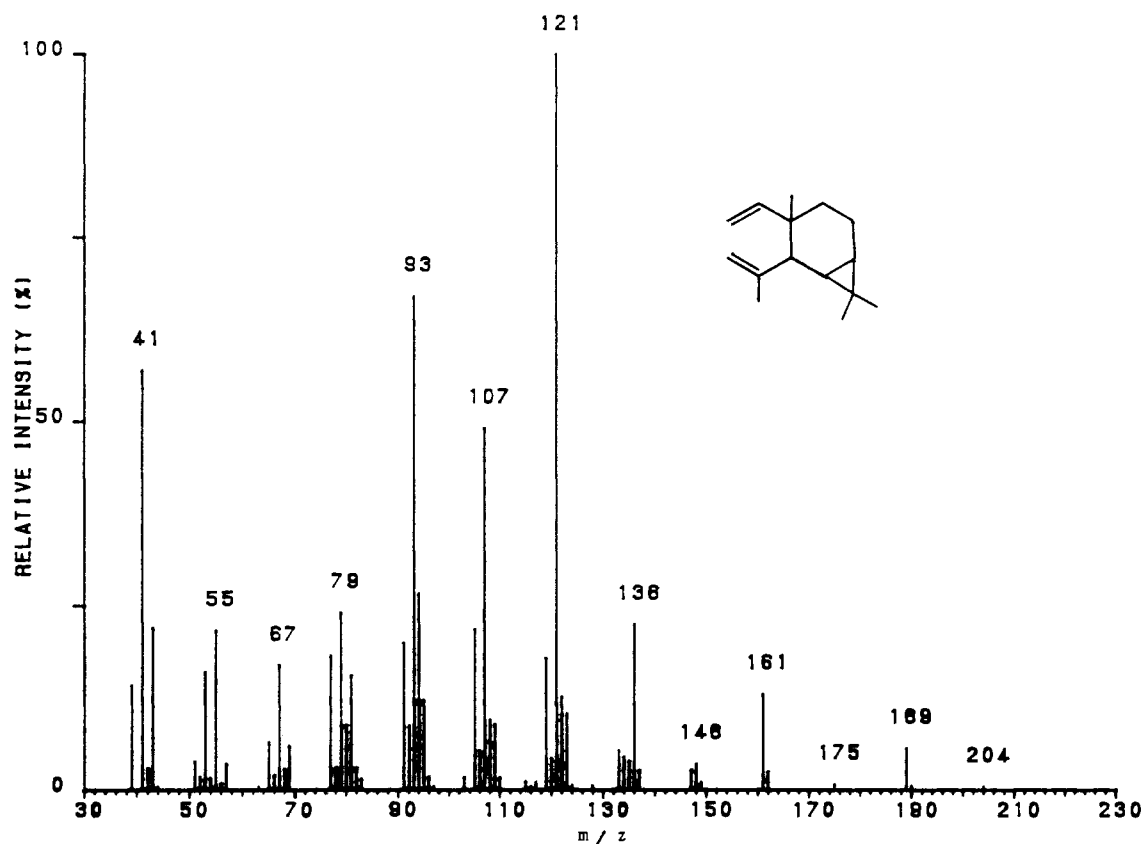
Figure 2. Mass spectrum (a) and gas-phase IR spectrum (b) of peak 35 identified as bicyclogermacrene.

its content (3.54%) is higher than the  $\beta$ -caryophyllene concentration (2.37%).

The isomers  $\gamma$ -cadinene and  $\gamma$ -muurolene were identified on the basis of their relative Kovats indices (Jennings and Shibamoto, 1980) as their respective mass spectra and gas-phase infrared spectra could not be differentiated. These cadinene group compounds, also with  $\delta$ -

cadinene, could be formed by thermal isomerization from germacrene D (Yoshihara et al., 1969).

Mass spectra and IR spectra of aromadendrene and alloaromadendrene were also hardly distinguishable. Their respective identities, however, were unambiguously assigned by comparison with commercially available products. Ledene was characterized in only one variety, Bosk-



**Figure 3.** Mass spectrum of the product formed by thermal isomerization in the hot GC injector from bicyclogermacrene and tentatively identified as bicycloelemene.

oop Giant. This sesquiterpene and its isomer viridiflorene are structurally related to aromadendrene and *allo*-aromadendrene. These four compounds could be derived from bicyclogermacrene after thermal isomerization (Nishimura et al., 1969).

While the hydrocarbon fraction is the major part of the oils, it does not explain their blackcurrant odor. Monoterpenes afford essentially green and resinlike notes (Latrasse, 1983) common to all the oils under investigation. However, some sesquiterpenes could be considered as flavor contributors. Thus, germacrene B was recently characterized as an important constituent of lime peel oil (Clark et al., 1987). The same could be suspected for the labile bicyclogermacrene, particularly abundant in the strongly aromatic variety Noir de Bourgogne. However, by sniffing the GC effluent of the hydrocarbon fraction of this cultivar, only two peaks out of the detected sesquiterpenes had any odor (described as spicy), and corresponded to  $\beta$ -caryophyllene and *allo*-aromadendrene.

**Oxygenated Fraction.** The oxygenated compounds content varied from 5 to 10% of the oils, depending on the cultivars. However, these polar fractions contained the most odorous volatile compounds and exhibit the characteristic blackcurrant odor (Latrasse, 1983).

The oxygenated fractions were characterized essentially by mono- and sesquiterpene alcohols and oxides and by monoterpene acetates.

The major compounds were terpinen-4-ol (from 0.40 to 4.51%), spathulenol (from 0 to 2.71%), caryophyllene oxide (from 0.18 to 1.50%), and bornyl acetate, with a relative amount varying from trace to 2.00%, but present essentially in those cultivars related to the selected hybrid Andega (Noir de Bourgogne  $\times$  Ojebyn).

Despite its relative high abundance in many varieties, terpinen-4-ol possesses a rather low but unpleasant ter-

pene-like and rootlike odor (Latrasse et al., 1982). Spathulenol, on the other hand, was considered as an important compound with a conifer-like note (Le Quéré and Latrasse, 1986), and it is found in higher amounts in aromatic varieties, such as Noir de Bourgogne.

Other abundant compounds in these fractions were *p*-mentha-1,5-dien-8-ol, *p*-cymen-8-ol, isospathulenol, citronellyl acetate, and  $\alpha$ -terpinyl acetate. These oxygenated compounds represented from 3.40 to 5.46% of the total oils.

*Cis* and *trans* isomers of *p*-menth-2-en-1-ol were identified on the basis of published Kovats indices and mass spectrum (Nykanen, 1986a). This also applied to the identification of *trans*- and *cis*-piperitol, previously characterized in blackcurrant buds oil (Kerslake and Menary, 1985). The identification of criptone was based on the perfect match of the eight most abundant peaks of the mass spectrum with published data (*Eight Peak Index of Mass Spectra*, 1974) and that of umbellulone on the basis of a published spectrum (Héthelyi et al., 1987).

The most important compounds for the aroma of the oils (Latrasse et al., 1982; Latrasse, 1983) were present in the polar fractions, even though they were found in very small amounts. Thus, the sulfur compound 4-methoxy-2-methyl-2-mercaptobutane (peak 1, Figure 1) responsible for the characteristic catty note was found in small amounts varying from trace to 0.04%, the highest level, however, being found in aromatic varieties (eg Noir de Bourgogne, Burga, Royal de Naples). Concentration of eucalyptol (1,8-cineole), important for its characteristic balsamic note, varied from trace to 0.01% of the total oils, and 1-octen-3-ol, with its mushroom odor, was only found in trace amount. More abundant in these polar fractions, monoterpene alcohols, such as linalool or citronellol, generally give off typical floral notes, while sesquiterpene alcohols and oxides, such as spathulenol,

caryophyllene oxide, or isospathulenol, afford conifer-like odors. Monoterpene acetates have been found particularly important for their characteristic floral or lemony notes.

Finally, this study on the chemical composition of the essential oils of blackcurrant buds allowed us to identify 26 compounds not previously reported, among which two discriminative sesquiterpene hydrocarbons, bicyclogermacrene and germacrene B, were precisely characterized. Moreover, we could confirm our previous chemotypic studies and extend the classification by including the less abundant polar compounds (Latrasse et al., 1988). A detailed chemosystematic study will be published later. The olfactory properties of the polar fractions were also considered, as they contained major odorous compounds affording typical blackcurrant aroma. However, some identification work still has to be done, as some of these compounds remained unknown.

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**Registry No.**  $\alpha$ -Thujone, 2867-05-2;  $\alpha$ -pinene, 80-56-8; camphene, 79-92-5; sabinene, 3387-41-5;  $\beta$ -pinene, 127-91-3; myrcene, 123-35-3; 4-carene, 29050-33-7;  $\alpha$ -phellandrene, 99-83-2; 3-carene, 13466-78-9;  $\alpha$ -terpinene, 99-86-5; *m*-cymene, 535-77-3; *p*-cymene, 99-87-6; limonene, 5989-27-5;  $\beta$ -phellandrene, 555-10-2; (*Z*)-ocimene, 27400-71-1; (*E*)-ocimene, 27400-72-2;  $\gamma$ -terpinene, 99-85-4; *trans*-sabinene hydrate, 17699-16-0; terpinolene, 586-62-9; *cis*-sabinene hydrate, 15537-55-0; *trans*-*p*-menth-2-en-1-ol, 29803-81-4; *cis*-limonene 1,2-epoxide, 13837-75-7; *cis*-*p*-menth-2-en-1-ol, 29803-82-5; *p*-mentha-1,5-dien-8-ol, 1686-20-0; terpinen-4-ol, 562-74-3; *p*-cymen-8-ol, 1197-01-9;  $\alpha$ -terpineol, 98-55-5; citronellol, 106-22-9; linalyl acetate, 115-95-7; bornyl acetate, 76-49-3;  $\delta$ -elemene, 20307-84-0; citronellyl acetate, 150-84-5;  $\alpha$ -terpinyl acetate, 80-26-2;  $\beta$ -elemene, 515-13-9;  $\beta$ -caryophyllene, 87-44-5;  $\gamma$ -elemene, 30824-67-0;  $\alpha$ -humulene, 6753-98-6; *allo*-aromadendrene, 25246-27-9; germacrene D, 23986-74-5; bicyclogermacrene, 24703-35-3;  $\delta$ -cadinene, 483-76-1; germacrene B, 15423-57-1; spathulenol, 6750-60-3; caryophyllene oxide, 1139-30-6; humulene epoxide, 19888-33-6; isospathulenol, 88395-46-4; *p*-mentha-1,3,8-triene, 18368-95-1; *allo*-ocimene, 673-84-7;  $\alpha$ -copaene, 3856-25-5;  $\beta$ -guaiane, 88-84-6;  $\beta$ -cubebene, 13744-15-3; aromadendrene, 72747-25-2;  $\alpha$ -gurjunene, 489-40-7;  $\gamma$ -muurolene, 30021-74-0; ledene, 21747-46-6;  $\gamma$ -cadinene, 39029-41-9; viridiflorene, 21747-46-6; 4-methoxy-2-methyl-2-mercaptobutane, 94087-83-9; 1-octen-3-ol, 3391-86-4; 1,8-cineole, 470-82-6; linalool, 78-70-6; *p*-mentha-1,3-dien-8-ol, 82538-84-9; *m*-cymen-8-ol, 5208-37-7; ciptone, 500-02-7; *trans*-piperitol, 16721-39-4; *p*-menth-2-en-7-ol, 72687-68-4; *cis*-piperitol, 16721-38-3; umbellulone, 24545-81-1; terpinen-4-yl acetate, 4821-04-9; neryl acetate, 141-12-8; geranyl acetate, 105-87-3;  $\alpha$ -cadinol, 481-34-5; 6,10,14-trimethyl-2-pentadecanone, 502-69-2.

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## Identification of Some Antibacterial Constituents of New Zealand Manuka Honey

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Some components responsible for the exceptionally high antibacterial activity of manuka honey were isolated by testing fractions of the honey for activity against *Staphylococcus aureus*. An ethanol-ether extract of the honey was separated by preparative-layer chromatography and the fractions thus obtained were assessed for antibacterial activity. One fairly homogeneous fraction was identified as methyl 3,5-dimethoxy-4-hydroxybenzoate (methyl syringate, **1b**). Combined gas chromatography-mass spectroscopy indicated the presence of this compound in some of the other antibacterial fractions together with methyl 3,4,5-trimethoxybenzoate (**1c**) and 3,4,5-trimethoxybenzoic acid (**1a**). Authentic specimens of 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid, **1d**) and 3,4,5-trimethoxybenzoic acid (**1a**) and their methyl esters were tested against *S. aureus*. The acids and, to a lesser extent, methyl syringate were found to possess significant antibacterial activity.

The antibacterial property of honey has long been recognized in vivo (Aristotle, 350 B.C.) and more recently in vitro (Sackett, 1919), but little work has been directed toward identifying the constituents responsible for this property. The antibacterial activity was originally thought to be due the high osmolarity of honey, but some activity persisted after dilution. Dold et al. (1937) termed this activity "inhibine" and found it to be labile to light and heat. Other workers (White et al., 1962; White and Subers, 1963) concluded that inhibine was hydrogen peroxide generated by the action of the glucose oxidase in honey; however, it was found (White and Subers, 1963) that some honey samples had antibacterial activity in excess of that which could be accounted for by the action of hydrogen peroxide alone. Also, Adcock (1962) found antibacterial activity to persist after the removal hydrogen peroxide by the addition of catalase.

Other authors have suggested that there may be additional antibacterial substances in honey. Antibacterial activity was found to be extractable with ethanol, acetone, and ether (Vergé, 1951; Schuler and Vogel, 1956;

Lavie, 1960). Gonnet and Lavie (1960) have reported the antibacterial extractives to be stable to light and reasonably stable to heat, while Lavie (1963) noted that some of the antibacterial substances recovered from an ether extract were volatile at 95 °C. More recently, Mladenov (1974) has reported that honey contains volatile, heavy-volatile, and nonvolatile antibacterial substances, while Dustman (1979) also noted the existence of antibacterial activity that was not due to glucose oxidase activity or the high osmolarity; however, he was of the opinion that the latter activity was only a minor portion of the total activity.

We consider it likely that the differences in opinion on the significance of the additional antibacterial activity (i.e., that not due to hydrogen peroxide or the high osmolarity) result from the differences that exist in the amount of this activity. Molan and Russell (1989) found that for a range of New Zealand honey samples the additional activity varied from nil in some samples, to almost the whole of the activity in other samples. They also noted a close correlation existed between the level of additional antibacterial activity and the overall antibacterial activity of individual honey samples. Because of this finding we undertook to assess the antibacterial activity of the organic substances present in the extractives of manuka honey, a honeytype known to possess substantial additional antibacterial activity (Molan and Russell, 1989).

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