Aroma Constituents of Blackcurrant Buds *(Ribes nigrum L.)*

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The classic use of blackcurrant bud absolute is to reinforce and modify natural or artificial blackcurrant flavour;[1] more recently it has found applications in fragrances, especially in the scenting of lipsticks.[2]

Early work on oil composition was carried out by French workers who prepared a nearly colourless oil by benzene extraction followed by steam distillation.[3,4] Later, a number of major components, mainly monoterpenes, were identified using Kovat's retention indices.[5,6]

In an important contribution Williams undertook the first reported organoleptic assessment of blackcurrant bud oil.[7] The majority of odour comments were characteristic of what one would expect from terpenes, being musty, pine-like or reminiscent of turpentine in the lower boiling point region, and spicy aromatic in the higher boiling point region. No particular region could be associated with the 'catty' blackcurrant aroma, although some peaks in the terpene hydrocarbon region did have a minty character. Other peaks had green and cucumber aromas, both of which Williams considered could contribute to the 'catty' note of the buds. One of the high boiling point regions was associated with the heavy, sweet smell of commercial blackcurrant flavours.

Latrasse and Lantin in renewed investigations demonstrated that the composition of the essential oil is a discriminative feature characteristic of each cultivar.[8,9] Recently another report states that the monoterpenes present in the volatile portion of the absolute cannot account for the typical and potent aroma of blackcurrants.[10] Further, these researchers found that the monoterpene fraction, when isolated by liquid chromatography lacked the characteristic odour completely. They detected this note clearly in the more polar and extremely complex mixture eluted after beta-caryophyllene.

In a preliminary communication some twelve new components in blackcurrants were reported and data presented for twenty-five unknowns.[11] The catty note was not isolated and an incomplete aroma profile was presented.

Experimental

The plants used, variety White Bud, were from a commercial farm in Southern Tasmania. The buds were macerated in petroleum ether (BP40-60°C) in a stainless steel blender and extracted in a rotating drum overnight. The solvent was drawn off and removed under reduced pressure at 30°C. A dark green resinous concrete with a

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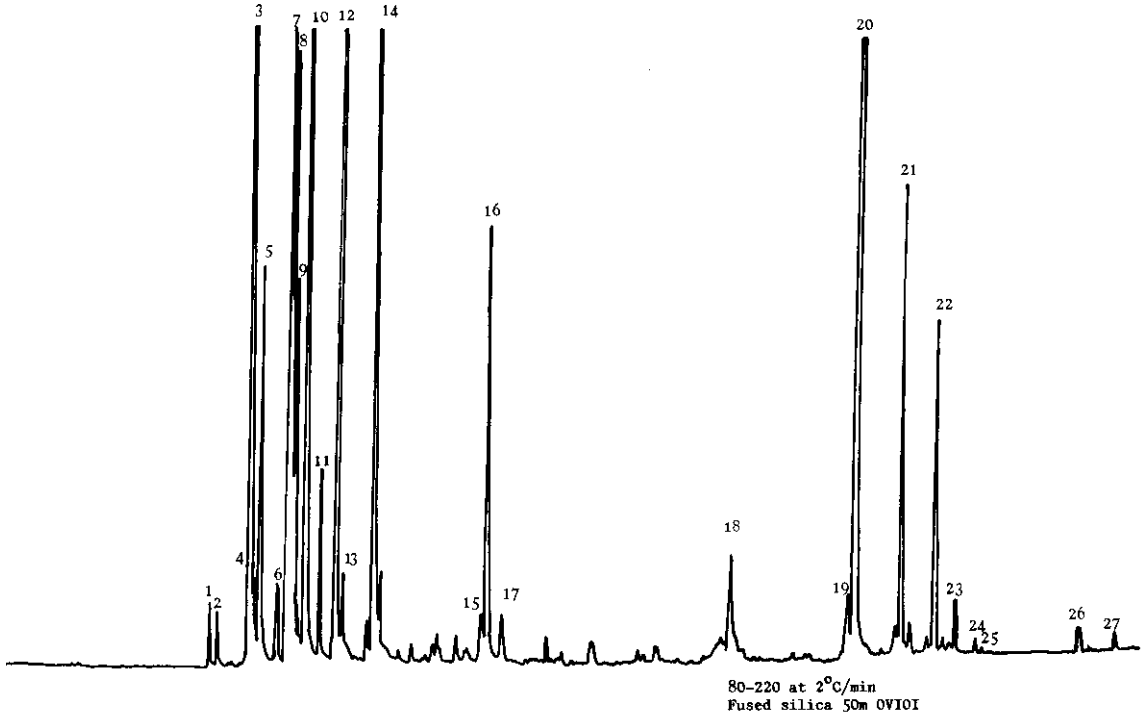

Figure 1. GC Trace of Blackcurrant Bud Oil

strong blackcurrant aroma was obtained. Some samples were prepared by extraction with liquid carbon dioxide in a commercially available Soxhlet extractor.[12] A vacuum distillate was prepared from the concrete using liquid nitrogen cold traps at a vapour pressure of 0.7 mmHg.

The silica gel was prepared for chromatography as previously described.[11] A petroleum ether extract of the concrete was applied to the column.

Fractions were eluted with a series of 20 ml volumes of three diethyl ether/pentane mixtures (5%, 10% and 15%), followed by 30 ml each of 25% and 50% diethyl ether in pentane. The volume of the first two fractions collected was 10 ml, all succeeding ones were 5 ml. The solvent was removed under a gentle stream of nitrogen to concentrate the samples. The fractions were then examined by gas chromatography and mass spectrometry. The vacuum distilled oil was separated by the same procedure.

A second liquid-solid chromatography method using Florisil as a packing was also developed to confirm results obtained on the silica gel. The Florisil was activated by ignition at 600°C for two hours using a furnace oven, then allowed to cool in the oven overnight. Before use it was reactivated by heating for two hours at 130°C and then stored in a dessicator.

Two ml of blackcurrant vacuum distillate was introduced to a 350 mm x 26 mm LKB column packed with Florisil in petroleum ether. Elution was started at a flow rate of 3 ml/min. Fractions were eluted with 1500 ml petroleum ether, followed by 600 ml diethyl ether and finally 300 ml dichloromethane. Fractions were collected as 100 ml samples and reduced in volume under a slow stream of nitrogen before injection.

A satisfactory procedure to separate blackcurrant bud oil into oxygenated monoterpenes, monoterpene hydrocarbons and sesquiterpene hydrocarbons was developed using an octadecylsilane-bonded silica phase (Radial-PAK μ Bondapak C18, 10μ particle size). The HPLC system consisted of a Waters Associates radial compression module (RCM-100), a dual solvent delivery

Table I. Identification of Components in Blackcurrant Bud Oil

Peak Number	Name	% Peak Area	Component not previously identified**	Chromatogram peak number (Figure)	Retention Indices I_{120}	I_{140}	I_{160}	$I_{Programmed}$	Source for Identification**
1	acetic acid	-	BF						4
2	isobutanol	-	F						4
3	n-butanol	-	F						4
4	pentan-2-ol	-	F						4
5	2 methyl butan-1 -ol	-	N						4
6	unknown alcohol (1)	-	U						4
7	unknown alcohol (2)	-	U						4
8	unknown alcohol (3)	-	U						4
9	unknown alcohol (4)	-	U						4
10	xylene isomers	-	F						7,11
11	tricyclene	0.01	N						3
12	alpha thujene	0.38	F	1	928	929		928	1
13	alpha pinene	0.33	BF	2	940	941		937	1
14	unknown MW119 (5)	tr	U						10
15	unknown MW120 (6)	tr	U						10
16	benzene	tr	F						11
17	benzaldehyde	tr	F						11
18	propyl benzene	tr	N						10
19	iso propyl benzene	tr	N						10
20	beta thujene	0.04	N						5
21	1-oct-en-3-ol	0.03	BF						11
22	unknown MW136 (7)	tr	U						6
23	1-ethyl 2 methyl benzene	0.03	N						6
24	sabinene	15.44	BF	3	957	970		971	1
25	beta-pinene	0.71	BF	4	972	981		977	1
26	1,2,3 trimethyl benzene	tr	N						10
27	myrcene	2.81	BF	5	990	993		984	1
28	unknown MW136 (8)	tr	U						6
29	unknown MW120 (9)	tr	U						9,10
30	1 methyl 2 ethyl benzene	tr	F						9,10
31	alpha phellandrene	0.69	BF	6	1001	1001		1000	1
32	unknown MW136 (10)	tr	U						5
33	unknown MW136 (11)	tr	U						5
34	delta-3-carene	12.65	BF	7	1008	1012		1010	1
35	alpha terpinene	3.90	BF	8	1011	1014		1014	1
36	p-cymene	2.64	BF	9	1013	1019		1017	1
37	beta-phellandrene	3.25	BF	10	1028	1030		1023	1
38	limonene	3.25	BF	10	1029	1036		1026	1
39	cis beta ocimene	1.26	BF	11	1033	1040		1029	1
40	trans beta ocimene	6.75	BF	12	1036	1057		1041	1
41	3,5,5 trimethyl n-hexanol	tr	N						11
42	2-ethyl-hexanol	tr	F						5,12
43	gamma terpinene	0.82	BF	13	1053	1061		1054	1
44	cymenene	0.02	N		1059	1090		1060	1
45	unknown MW154 (12)	0.02	U						5
46	linalool	0.28	BF					1078	1
47	alpha terpinolene	11.63	BF	14	1008	1097		1083	1
48	non-an-2-one	1.11	N					1089	1
49	unknown MW154 (13)	tr	U						5
50	unknown MW154 (14)	tr	U						11

Table I. (continued)

Peak Number	Name	% Peak Area	Component not previously identified**	Chromatogram peak number (Figure)	Retention Indices I_{120}	I_{140}	I_{160}	$I_{Programmed}$	Source for Identification**
51	unknown (15)	tr	U						11
52	unknown (16)	tr	U						11
53	unknown MW152 (17)	0.35	U		1132	1137			5,7,11
54	unknown MW152 (18)	tr	U						4
55	unknown MW152 (19)	tr	U						4
56	unknown MW152 (20)	tr	U						4
57	unknown MW154 (21)	tr	U						4
58	unknown MW182 (22)	tr	U						7
59	cis-p-menth-2-ene 1,8 diol	tr	N						4
60	menthone	0.24	N						7
61	unknown MW182 (23)	tr	U						7
62	napthalene	0.25	N						7
63	terpinen-4-ol	0.51	BF	15	1166	1172		1165	1
64	alpha terpineol	3.78	BF	16	1172	1178		1168	1
65	p cymen-8-ol	0.53	F						4
66	trans piperitol	0.11	F	17	1180	1186		1179	1
67	sabinene hydrate	0.06	N						4
68	unknown MW152 (24)	tr	U						4
69	unknown MW152 (25)	tr	U						4
70	unknown MW154 (26)	tr	U						4
71	unknown MW150 (27)	tr	U						7
72	unknown MW182 (28)	tr	U						7
73	unknown MW182 (29)	tr	U						7
74	carvone	0.21	F						11
75	unknown MW182 (30)	tr	U						7
76	unknown MW180 (31)	tr	B						7
77	unknown MW180+182 (32)	tr	U						7
78	unknown MW180 (33)	tr	U						7
79	unknown MW168 (34)	tr	U						5
80	unknown MW182 (35)	0.12	U					1237	2,12
81	citronellyl formate	0.17	U					1241	7
82	bornyl acetate	0.17	B		1271				6,7
83	2-undecanone	tr	N						7
84	4-terpinyl acetate	0.78	F		1331	1332			6,7
85	beta terpinyl-acetate	1.87	N	18		1336		1336	7
86	citronellyl acetate	0.01	BF						7
87	geranyl acetate	0.03	F					1353	7
88	methyl undecanoate	0.02	N						7,11
89	alpha copaene	0.08	B						2
90	unknown MW204 (36)	0.05	U						4
91	unknown MW204 (37)	0.10	U						4
92	beta elemene	0.84	B	19		1414	1428	1412	1
93	beta caryo-phyllene	12.39	BF		1413	1427	1439	1421	1
94	unknown MW204 (38)	0.06	U			1431			7
95	unknown MW204 (39)	0.30	U		1427	1441	1455		7
96	humulene	3.79	BF	21	1440	1457	1468	1454	1
97	alloaromadrene	0.25	N		1445	1460	1482	1461	4,5
98	unknown MW204 (40)	0.02	U						5
99	unknown MW204 (41)	0.13	U						7

Table I. (continued)

Peak Number	Name	% Peak Area	Component not previously identified**	Chromatogram peak number (Figure)	Retention Indices I_{120}	I_{140}	I_{160}	$I_{Programmed}$	Source for Identification **
100	Germacrene D	2.61	N	22	1464	1478	1491	1479	2,4,5
101	gamma elemene	0.83	F	23		1505		1493	1
102	unknown MW204 (42)	0.10	U						6
103	gamma cadinene	0.10	N						4,5
104	beta cadinene	0.08	N						4,5
105	unknown MW204 (43)	tr	U						5
106	beta elemene alcohol?	0.20	N	24		1513	1517	1516	7
107	gamma elemene alcohol?	0.07	N	25			1522		4,7
108	caryophyllene epoxide	0.25	N	26			1574	1574	1,4
109	unknown MW204 (44)	tr	U						8
110	humulene epoxide	0.19	N	27			1580		1,4
111	unknown (45)	-	U						8
112	unknown (46)	-	U						8
113	unknown (47)	-	U						8
114	unknown MW204 (48)	-	U						7
115	unknown MW220 (49)	-	U						4
116	unknown MW220 (50)	-	U						4
117	unknown MW220 (51)	-	U						4
118	unknown MW220 (52)	-	U						4
119	unknown MW220 (53)	-	U						4
120	unknown MW220 (54)	-	U						4
121	unknown MW250 (55)	-	U						8
122	unknown MW250 (56)	-	U						8
123	unknown MW286 (57)	-	U						4

Key to Table I.

** source for identification
1 blackcurrant concrete
2 vacuum distillate
3 concrete headspace
4 liquid carbon dioxide extract
5 liquid carbon dioxide
6 silica gel chromatography Fraction 12
7 silica gel chromatography Fraction 19
8 silica gel chromatography Fraction 24
9 Florisil chromatography Fraction 8
10 Florisil chromatography Fraction 13
11 Florisil chromatography Fraction 17
12 HPLC Fraction 1

** component
B previously identified in buds
F previously identified in fruit
N newly identified
U unknown

pump system (model 6000A) and a universal injector (model UK6). Detection was at 214 nm using a discrete multiwavelength absorbance detector (model 440) fitted with an extended wavelength module. The mobile phase consisted of methanol and water, which allowed for low UV monitoring and resulted in a good separation of the three groups.

The solvent programme consisted of stepwise elution with methanol/water 70:30 for twenty-five minutes and then pure methanol for fifteen minutes at a flow rate of 4 ml/min.

Gas chromatography (GC) analysis of oil samples and chromatography fractions was conducted using a Pye Unicam Series 104 chromatograph fitted with an FID detector. Initially this chromatograph was connected to a Pye Unicam PD88 integrator and a Rikadenki chart reorder. However, in later work a Sigma 10 (Perkin Elmer) data station was used to collate the information.

For routine analysis and aroma profile identification a 50 m x 0.5 mm ID OV101 SCOT capillary column was employed. Operating conditions were as follows: carrier gas was nitrogen at a gas velocity of 153 cm/sec, air flow rate 600 ml/min and hydrogen flow rate 60 ml/min. At the effluent end of the column, nitrogen was used as a make-up gas at a flow of 60 ml/min. The column oven temperature was programmed from 80 to 220°C at 5°C/min.

For determination of Kovat's retention indi-

Table II. Aroma Sensations Detected with Blackcurrant Vacuum Distillate

Aroma	Peak	Identity
1** steely spicy		
clear hollow	12	alpha thujene
pine	13	alpha pinene
sweet		
camphorous		
dull woody		
pine like	24	sabinene
clear-resin	25	beta pinene
2** blackcurrant fruit		
** cat's urine		
unpleasant sulphur		
sweet pine	31	alpha phellandrene
wet wood		
sweet pine	34	delta-3-carene
dry wood		
eucalyptus		
musty		
musty		
bitter sweet		
lemon	37	beta phellandrene
citrus	38	limonene
pine resin	40	trans-beta-ocimene
sweet/pine	43	gamma terpinene
musty		
dry woody	47	alpha terpinolene
sweet floral		
3** blackcurrant fruit		
musty pine		
citrus		
taint		
lemon		
woody		
sugary		
compost	62	terpinen-4-ol
damp soil	63	alpha terpineol
earthy		
lemon		
woody		
flatulent		
rich sweet		
jam like		
woody		
sharp wood		
sweet		
4** blackcurrant fruit		
flatulent		
fungi		
sweet		
damp		
musky	92	beta caryophyllene
flower blossom		
citrus/lemon/sharp		
wood shavings		
sickly sweet	95	humulene
floral		
sweet fruity	99	germacrene-D
damp wood		
sharp wood		
sharp, acidic, citrus		
jam burnt		
sweet antiseptic		
5** blackcurrant fruit		
woody antiseptic		

ces,[13,14] a 50 m x 0.2 mm ID Fused Silica OV101 column was used with nitrogen, gas velocity 51 cm/sec, as a carrier. An injection volume of 0.5 μl was sampled from all eluted fractions and 0.02 μl from all concentrated oil extracts.

A dual detection system utilizing a Hewlett Packard 583A gas chromatograph fitted with an FID and a photometric detector was also employed. The column used was a SCOT 30 m SP2100, with a helium carrier gas velocity of 51 cm/sec and make-up gas nitrogen. The injector temperature was 230°C with detector temperatures 250°C and 230°C for FID and photometric detectors respectively. The oven temperature was held at 60°C for 5 minutes, then programmed 60-175°C at 5°C/min, then held for 2 minutes before being programmed to 190°C at 5°C/min. This system was employed to examine the five regions of organoleptic interest for any sulphur containing compounds.

In order to examine the headspace above samples a Pye Unicam Headspace analyser (model 4750) was connected to the injection end of an OV101 SCOT glass capillary column (50 m x 0.5 mm ID).

The basic temperature programme of 80-220°C at 5°C/min was used and later modified to start the programme at various temperatures from 50 to 80°. The headspace analyser was also connected to the combined gas chromatography/mass spectrometry facility which consists of a Pye Unicam 204 chromatograph directly coupled, via a glass-lined steel tube (heated at 200°C), to a VG Micromass 70/70F mass spectrometer. A fused silica OV101 column was used with a hydrogen carrier flow rate of 1.5 ml/min. The spectrometer is a high resolution, double focussing model operated at an ionizing energy of 70 eV, a 4 KV accelerating voltage and an ion source temperature of 200°C. The range M/Z 300 to 20 was scanned exponentially downward at 1s/decade, resulting in a full mass spectrum every two seconds. The data was stored in a VG2035 data system. Spectra were enhanced by background subtraction with generation of reconstructed spectra and gas chromatograms where necessary.[15] Gas chromatograms were represented by Total Ion Current (TIC) changes with time. Library search facilities were also available using a seven major peak search capability.

Results

The combined GC/MS facility was used to examine blackcurrant oils in a variety of forms—as concretes, vacuum distillates, column chromatography fractions, HPLC fractions and liquid carbon dioxide extracts. The information obtained from these extracts is contained in Table I and figure 1. Identification of component

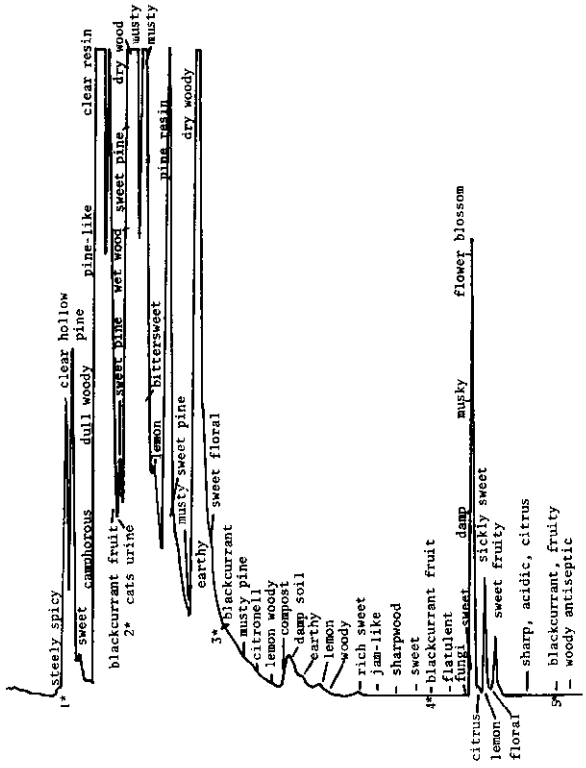

Figure 2. Aroma profile Tasmanian blackcurrant bud oil 50m SCOT OV 101. 80-220 at 5°C/min.

peaks was made by comparison with published work using VG data systems library search capabilities.[16-19]

A total of one hundred and twenty-three components has been detected in the blackcurrant oil, of which sixty-six have been positively identified and are named in Table I. High resolution GC/MS has enabled formulae and structural information to be derived for some unknown components; this is reported in the literature.[20] Of the sixty-six components which have been positively identified, some twenty-three are compounds not previously identified in blackcurrant fruit or bud oils as reported in the literature also reviewed elsewhere.[20]

The outcome of attempts to relate odours to compounds eluting from the gas chromatography column are presented in Table II and figure 2. The study has indicated that while the aroma profile is complex, five regions have been identified as important in the overall blackcurrant aroma impression. The first region that has been identified retains a steely spicy note very reminiscent of the French CAL Cassis absolute. The second region contains the characteristic "catty" note with an after impression of blackcurrant fruit. The catty odour is extremely intense, completely overriding the previously dominant pine/resin aromas. The other three regions give impressions of blackcurrant fruit alone.

Discussion

In this present study a variety of liquid chromatography techniques were used in order to isolate and identify the catty note of blackcurrants. Despite silica gel being confirmed as effective in separating hydrocarbons from oxygenated compounds, as shown by other workers,[21,22] the catty note was not eluted. Since none of the fractions possessed this catty aroma, it appears that the precautions taken to deactivate and neutralize the silica gel were not sufficient to ensure this compound's stability. The compound responsible for this catty aroma, it is argued, is therefore very liable and readily undergoes chemical rearrangement.

An alternative hypothesis exists; the catty note is the result of two or more compounds which have separated into different fractions, hence the loss of aroma is readily explained. There is a lack of confirmational evidence for this proposal, and indeed the study provides circumstantial evidence suggesting the involvement of only a single compound.

Florisil, which has been preferred to silica gel for difficult separations of terpene constituents,[23] was also unable to elute the catty note, thereby supporting the contention that this aroma compound is extremely labile. Although other workers suggest that isomerization processes can be avoided by using purification and deactivation procedures,[23,24] this current work demonstrates such is not the case when the catty component is in contact with polar absorbants.

The failure to achieve elution of the catty note from a polar absorbant suggested the need to attempt a reverse phase separation.

An effective HPLC method for prefractionation of monoterpene and sesquiterpene hydrocarbons from the oxygenated compounds was developed, confirming the results of Kubeczka.[25] This method enabled the catty aroma to pass through the column unchangad in one small fraction; suggesting that the catty note is a single component that undergoes some chemical change on polar absorbants. It is important to realize that the polarity system was reversed with a non-polar absorbant (Bondpak C18) and a highly polar solvent (methanol/water), as opposed to the previous polar absorbant (silica gel) and non-polar solvent (hexane).

Effluent trapping of gas chromatographic samples was not found to be useful, other than as a confirmatory technique for components identified by other separatory procedures. This was due to two factors: firstly, the resolving power of the glass capillary column and secondly, the nature of the peaks of real interest. The complexity of the blackcurrant aroma determined that minor peaks in the chromatogram were of greatest interest. The resolving power of the glass column meant that some of these peaks were not separated adequately from major components. Also, the inability to load the column with samples large enough to enhance the peaks of interest sufficiently restricted the usefulness of trapping procedures. Prefractionation procedures, particularly by HPLC, improved this situation markedly; however, at this stage the combination of a fused silica column and the fast scan capabilities of the mass spectrometer made the trapping requirement redundant.

Headspace analysis was a useful technique in separating and identifying a number of early eluting components. These peaks were not apparent in routine gas chromatographic analysis of blackcurrant concretes due to the presence of residual solvent peaks. There is an extensive literature, some of which has been reviewed elsewhere,[20] which supports the results obtained in this study confirming the ability of headspace analysis to reliably reproduce the natural aroma. Importantly, the presence of these early components was confirmed by combined gas chromatography/mass spectrometry analysis of the liquid carbon dioxide extract; demonstrating the superiority of this extract in retaining the true natural aroma, free from solvent contamination.

Utilizing all these techniques, most of the compounds previously detected in blackcurrant bud oil were identified in the current work. However, the following components, delta-cadinene, citronellol, ethyl oleate, methyl palmitate, reported by Williams,[7] and sabinol and geraniol, reported by Glichitch and Igolen,[4] have not been identified in Tasmanian extracts.

Wide differences are reported in the literature concerning the relative percentages of components in blackcurrant buds. For example, Fridman et al.[6] reported limonene (23.91%) as the most abundant component, whereas Latrasse[5] noted that myrcene (34%) and caryophyllene (21.2%) were present in larger amounts than limonene (10.9%). Likewise, Williams[7] recorded, in extracts from mixed cultivars, that limonene (0.8%) was only of secondary importance to the major compounds, delta-3-carene (15%), beta-pinene (24%) and terpinolene (9%). In this cur-

rent study, sabinene (15.44%), delta-3-carene (12.65%), alpha terpinolene (11.63%) and beta caryophyllene (12.39%) were recognized as major components. Further, limonene (3.25%), beta pinene (0.71%) and myrcene (2.81%) were of lesser importance in the extracts studied.

Part of this present study reported elsewhere,[20] suggests that the major reason for such conflicting results is of genetic origin. This hypothesis has been supported by other workers.[7-9,26] In addition, the amount of oxidation that takes place during extraction or storage may also account for some of the reported compositional differences. While no evidence has been presented here to support this premise, it is known that monoterpenes in blackcurrant bud oils readily oxidise on exposure to air.[27] Likewise, Williams determined that estimates of limonene were found to vary with the degree of oxidation that occurred during the extraction process.

This attempt to relate odours to compounds eluting from the gas chromatography column revealed that the blackcurrant bud aroma is complex, with five regions of major interest. Aroma regions 3 to 5 possess blackcurrant fruit aromas and are, most likely, the basis of Andersson and von Sydow's claim that the characteristic blackcurrant note was localized in the high boiling point fraction.[28] Similarly, Williams[7] associated the heavy sweet smell of commercial blackcurrant flavours with the high boiling point region.

The catty aroma was not identified by Williams, but he suggests that peaks with green or cucumber aromas could contribute to this catty note. In addition, he reported difficulty in eluting the catty note from a packed Carbowax 20 M column. No such difficulty was encountered using a capillary OV101 column in this study. Improvements in column resolution and the use of a non-polar phase are the most likely reasons for this result. French workers have reported that the catty note passed through three columns of differing polarity (SF96, Carbowax 20 M and Pluronic L64) supporting the contention concerning improvements in column technology.[26]

Various sulphur-containing compounds with similar odours have been suggested as possibilities for the catty constituent.[29,30] These researchers associated a catty note with (+) menthon-8-thiol in Buchu oil, while one also presented a synthesis based on pulegone.[29] This compound was not detected in the current work although pulegone and related components menthone and cis-p-menth-2-ene 1,8 diol were detected. Indeed, despite the use of prefractionation techniques and a sulphur-specific gas

chromatographic detector, no sulphur-containing compound was elucidated.

The presence of pulegone and a compound of molecular weight 186 with similar mass spectral and gas chromatographic characteristics as p-menthon-8-thiol has been confirmed in blackcurrant buds;[31] but no mention was made of the aroma associated with the latter compound. The structural elements, $-C(CH_3)_2$-SH, of the keto thiol identified in Buchu have also been utilized for the synthesis of compounds possessing catty notes.[32,33]

In the most recent paper on blackcurrant fruit aromas it was reported that the important aroma constituents are methyl and ethyl butyrates, 1-8-cineole, diacetyl and a catty unknown.[26] None of the four named components were identified in this current study, which indicates distinct differences exist between the fruit and bud aromas.

Further, it is evident that the component responsible for the catty note is present only in a very small amount, has a very low odour threshold, and while the probability that it contains sulphur remains, its identity is still unknown. Future studies will centre on improving the HPLC separation and concentrating the fraction with this aroma.

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