# Aroma compounds of fresh blackberries (*Rubus laciniata* L.)

**Dimitris N. Georgilopoulos and Annie N. Gallois**

Laboratoire de Recherches sur les Aromes, Institut National de la Recherche Agronomique, B.V. 1540, F-21034 Dijon Cedex, France

**Über die Aromastoffzusammensetzung von Brombeeren (*Rubus laciniata* L.)**

**Zusammenfassung.** Die flüchtigen Aromastoffe von Brombeeren (*Rubus laciniata* L.), Thornless Evergreen, wurden durch kontinuierliche Flüssig-Flüssig-Extraktion und anschließender chromatographischer Vortrennung an Kieselgel isoliert. Mit kombinierter gaschromatographischer-massenspektrometrischer Analyse wurden 245 Komponenten, davon 212 zum ersten Mal, identifiziert. Die Inhaltsstoffe der Kieselgel-Fraktionen wurden sensorisch am Ende der chromatographischen Kolonne beurteilt. Die wichtigen Komponenten sind 2-Heptanol, p-Cymen-8-ol, 2-Heptanon, 1-Hexanol, α-Terpineol, Pulegon, 1-Octanol, Isoborneol, Myrtenol, 4-Terpineol, Carvon, Elemicin und Nonanal.

**Summary.** The volatile components of fresh cultivated blackberries (*Rubus laciniata* L.), Thornless Evergreen variety, were isolated by continuous liquid-liquid extraction and fractionated on a silica gel column. Analysis by coupled gas chromatography-mass spectrometry led to the identification of 245 compounds (212 for the first time). The constituents of the silica gel fractions were sensorially assessed by sniffing the chromatographic effluents. The aroma of fresh blackberries is mainly due to the presence of 2-heptanol, p-cymen-8-ol, 2-heptanone, 1-hexanol, α-terpineol, pulegone, 1-octanol, isoborneol, myrtenol, 4-terpineol, carvone, elemicine, and nonanal.

## 1 Introduction

Until recently, blackberries have been a marginal crop with no other use than for home made preserves. Recently the food industry has shown increasing interest in this berry. This has led to various attempts to create new cultivated varieties which would be organoleptically equivalent to the wild types, with good resistance and productivity.

However little attention has been paid to the flavour of blackberries. Only three papers have been published during the past twenty years [1–3], dealing with the flavour of commercial blackberry essence (Evergreen variety). Twenty-three compounds were identified. Among them, only 3,4-dimethoxy allylbenzene was considered to contribute to the characteristic flavour of blackberries.

The aim of this work was to obtain a detailed picture of the aroma of fresh cultivated blackberries.

## 2 Material and methods

### *2.1 Material*

Cultivated blackberries (Evergreen Thornless, a mutant derived from the Himalayas), collected in August 1983 and deep-frozen over a fluidised bed at −40 °C, were received from Holland and stored at −20 °C until used.

All solvents used were purified by distillation.

### *2.2 Preparation of juice and extraction*

The deep-frozen berries were slurried with purified methanol in a nitrogen atmosphere in a commercial Waring blendor; the slurry was centrifuged for 15 min at 3000 *g*. About 530 ml of clarified liquid, organoleptically similar to the fresh blackberry, was obtained from each kg of berries. The pH of this juice was 3.2.

The clarified liquid (6 × 530 ml) was continuously extracted in a liquid-liquid extractor (Quickfit-type) with 1 l of Forane 11 (trichlorofluoromethane, Atochem, Levallois, France) and then with 1 l of dichloromethane for 48 h for each solvent.

The extracts were pooled after drying over anhydrous sodium sulfate.

Pigments and waxes were eliminated by vacuum distillation, first at $10^{-2}$ Torr, then by cold finger distillation at $10^{-5}$ Torr [4]. After washing with a $NaHCO_3$ 10% aqueous solution (w/v) (3 × 10 ml) to remove acidic compounds, the neutral extracts were carefully concentrated to a small volume (200 µl) with Dufton-type columns [5].

### *2.3 Silica gel chromatography*

Liquid chromatography on silica gel was performed using a jacketed glass column (20 cm × 1 cm), cooled to 0 °C and packed with 7 g silica gel (Merck, 240–260 Mesh), previously dried (60 °C, 4 h in a vacuum oven) and rehydrated with 33% purified water according to Palmer [6].

*Offprint requests to:* A. Gallois

Z Lebensm Unters Forsch (1987) 184:374–380

The whole neutral extract (200 µl) was first separated into three 50 ml fractions by successively eluting with pentane, dichloromethane and diethyl ether.

The dichloromethane fraction was rechromatographed using a dichloromethane-pentane gradient. Three ml fractions were eluted with 15 × 20 ml of mixtures containing successively 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% of dichloromethane. Their odour was then described and adjacent fractions exhibiting similar odours were pooled. This resulted in twelve new fractions. All fractions were concentrated to 50 µl for further examination.

*2.4 Gas chromatography*

Gas chromatographic analyses were performed on a Girdel 300 apparatus (Delsi, Suresnes, France), equipped with a 30 × 0.32 mm (i.d.) fused silica capillary column DB5 (J&W Scientific Inc., Rancho Cordova, CA). The film thickness was 1 µm. The hydrogen carrier gas flow rate was 37.5 cm/s. The oven temperature was programmed from 45 °C to 220 °C at a rate of 2 °C/min. Injector and flame ionization detector temperatures were maintained at 260 °C and 270 °C respectively. Retention indices were determined using these conditions.

Peak areas were recorded with a Minigrator integrator (Spectra Physics, Orsay, France). The percent occurrence of each compound was calculated with reference to the total area of the first 90 min of the chromatogram, excluding the solvent peak area.

*2.5 Mass spectrometry*

Gas chromatographic peaks were identified by GC-MS using a Nermag R-10-10 (Nermag, Rueil Malmaison, France) coupled with a Girdel 31 gas chromatograph. The GC was equipped with a 60 m × 0.32 mm i.d. fused silica DB5 capillary column directly connected to the ion source. The operating GC conditions were the same as above. Mass spectra were recorded with an ion-source energy of 70 eV. The temperatures of the ion-source and of the interface were 150 and 280 °C respectively. The scanning rate was 0.8 s from 25 to 300 dalton.

Molecular weights of identified or still unknown compounds were confirmed by chemical ionization mass spectrometry using methane, with an ionizing energy of 90 eV, a source pressure of 0.3 Torr, a source temperature of 90 °C and a repeller tension of about 1 V.

*2.6 Infra-red analysis*

Infra-red spectra were recorded with a Bruker IFS 85 coupled with a Carlo-Erba 5160 GC equipped with a 30 m × 0.54 mm i.d. DB5 fused silica column (film thickness 1.5 µm). The column effluent passed through a heated transfer line to a 1.5 mm i.d. × 36 cm gold coated, heated light-pipe. A M.C.T. large band (4800–600 $cm^{-1}$) detector was used. The temperature of both gold plated light-pipe and transfer line as 200 °C. Twelve interferograms/s were averaged to three, which lead to 4 interferograms/s.

*2.7 Gas chromatographic odour assessment*

Column effluents, separated using the Girdel 300 described above, were split 1:1 between the FID and a sniffing-port through a microneedle valve (S.G.E., 1/16″ OD MNVT). The diverted effluent was mixed with a moistened air flow (60 ml/min). Each extract was sniffed twice. The odour quality of each compound was assessed and its odour intensity was estimated on a three-point scale.

## 3 Results and discussion

From the three fractions obtained by silica gel chromatography, the diethyl ether fraction was odourless and was not studied further. Acidic compounds previously separated were also discarded for the same reason. Table 1 lists all the aroma compounds identified in the pentane and dichloromethane fractions. The pentane fraction contained only hydrocarbons and exhibited a very weak odour. Among the 12 fractions obtained by rechromatographing the dichloromethane fraction on silica gel, only fraction 3 possessed a berry-like odour. This fraction contained mainly aldehydes and esters.

135 compounds were identified and 110 tentatively identified in the blackberry extracts. Thirteen compounds represented 67% of the total odorous profile. 2-heptanol was the major compound (43.6%), the other 12 being p-cymen-8-ol (3.72%), 2-heptanone (3.32%), 1-hexanol (3.05), α-terpineol (2.38%), pu-

**Table 1.** Volatile compounds identified and tentatively identified in the juice of fresh blackberries

| Compounds | %[a] | [OI][b] | RI[c] |
|---|---|---|---|
| *Hydrocarbons* | | | |
| Benzene | + | [0] | |
| Toluene | + | [0] | |
| o-Xylene | + | [0] | |
| *p*-Xylene | + | [0] | |
| Styrene | + | [0] | |
| *m*-Xylene | + | [0] | |
| α-Pinene[d] | + | [1] | 936 |
| Camphene[d] | + | [1] | 948 |
| β-Pinene[d] | + | [1] | 975 |
| α-Phellandrene[d] | + | [1] | 1008 |
| *p*-Cymene[d] | + | [1] | 1024 |
| Limonene[d] | + | [1] | 1044 |
| γ-Terpinene[d] | + | [1] | 1058 |
| α,*p*-Dimethyl styrene[e] | + | [0] | |
| 2-carene | + | [1] | |
| α-Terpinolene[d] | + | [1] | 1104 |
| α-Cubebene[d] | + | [0] | 1350 |
| δ-Cadinene[d] | + | [1] | 1525 |
| *Esters* | | | |
| Ethyl acetate[d, f, g] | + | [2] | |
| Diethyl carbonate[d, f] | + | [0] | |
| Methyl propanoate[d, f] | + | [1] | |
| Methyl isobutyrate[f] | + | [1] | |
| Methyl 2-methyl acrylate | + | [0] | |
| Ethyl propanoate[f] | + | [0] | |
| Methyl butanoate[d, f] | + | [1] | |
| Methyl crotonate[e] | + | [0] | |
| Methyl isopentanoate | + | [2] | |
| Butyl acetate[d, f] | 0.01 | [1] | 819 |
| Ethyl butanoate[d, f] | tr | [2] | 827 |
| Methyl pentanoate[d, f] | 0.11 | [1] | 831 |
| Ethyl 2-methyl butanoate[d, f] | 0.07 | [3] | 860 |
| Ethyl 3-methyl butanoate[d, f] | 0.32 | [1] | 864 |
| Isopentyl acetate[d] | 0.08 | [1] | 895 |
| Pentyl acetate[d] | 0.07 | [0] | 927 |
| Methyl hexanoate[d, f] | 0.17 | [1] | 936 |
| Dimethyl malonate | tr | [1] | 940 |
| Butyl butanoate[d] | tr | [1] | 997 |
| *trans*-2-Hexenyl acetate[d] | 0.30 | [2] | 1000 |
| Ethyl hexanoate[d, f] | 0.29 | [2] | 1002 |
| Hexyl acetate[d] | tr | [1] | 1016 |
| *trans*-3-Hexenyl acetate[d] | tr | [1] | 1018 |
| Methyl heptanoate[d] | tr | [1] | 1033 |
| Dimethyl succinate | tr | [0] | |
| Butyl pentanoate[d] | tr | [0] | 1068 |
| Diethyl malonate[d] | 0.56 | [0] | 1084 |
| Methyl octanoate[d] | tr | [1] | 1138 |
| Diethyl succinate[d] | tr | [1] | 1170 |
| Ethyl benzoate[d] | tr | [1] | 1178 |
| Ethyl octanoate[d, f] | tr | [1] | 1200 |
| Octyl acetate[d, f] | tr | [1] | |

**Table 1** (continued)

| Compounds | %[a] | [OI][b] | RI[c] |
|---|---|---|---|
| Methyl nonanoate[d] | 0.12 | [1] | 1229 |
| Ethyl salicylate | tr | [1] | |
| Dimethyl adipate[e] | tr | [0] | |
| Hexyl isobutanoate[e] | 0.21 | [0] | |
| Methyl decanoate[d] | 0.03 | [1] | 1329 |
| Ethyl decanoate[d, f] | 0.05 | [2] | 1398 |
| Ethyl cinnamate[d] | 0.12 | [1] | 1481 |
| Methyl dodecanoate[f] | 0.23 | [2] | 1532 |
| Ethyl 5-hydroxy hexanoate[e] | tr | [1] | |
| Ethyl dodecanoate[d] | 0.13 | [1] | 1596 |
| Methyl tridecanoate[e] | tr | [0] | |
| Trimethyl citrate[e] | tr | [1] | |
| Ethyl 5-hydroxy octanoate[e] | tr | [1] | |
| Methyl tetradecanoate | 0.70 | [2] | 1731 |
| Ethyl 5-hydroxy decanoate[e] | tr | [1] | |
| Benzyl benzoate[e] | 0.07 | [0] | 1789 |
| Ethyl tetradecanoate[d] | tr | [1] | 1796 |
| Methyl pentadecanoate[e] | tr | [0] | |
| Ethyl pentadecanoate[e] | tr | [1] | |
| Methyl hexadecanoate | tr | [0] | 1933 |
| Methyl 9-hexadecenoate[e] | tr | [0] | |
| Ethyl hexadecanoate[d] | tr | [1] | 1994 |
| Methyl heptadecanoate[e] | tr | [0] | |
| Methyl 9-octadecenoate[e] | tr | [0] | |
| Methyl 9,12,15-octadecatrienoate | tr | [0] | |
| Methyl 11,14,17-eicosatrienoate | tr | [0] | |
| *Total percentage* | 3.64 | | |
| *Aldehydes* | | | |
| Butanal[d] | + | [0] | |
| 3-Methyl butanal[d, f] | + | [0] | |
| Pentanal[d, f] | + | [2] | |
| Hexanal[d, f] | 0.53 | [3] | 803 |
| *trans*-2-Hexenal[d, f] | 0.31 | [2] | 863 |
| Heptanal[d, f] | + | [3] | 913 |
| Benzaldehyde[d, f] | 0.20 | [1] | 967 |
| *trans,cis*-2,4-Heptadienal[f] | 0.44 | [1] | 991 |
| Octanal[d, f] | 0.25 | [3] | 1004 |
| *trans,trans*-2,4-Heptadienal[d, f] | 0.13 | [1] | 1017 |
| Phenylacetaldehyde[d, f] | 0.21 | [1] | 1067 |
| *trans*-2-octenal[d] | 0.15 | [3] | 1069 |
| Nonanal[d, f] | 1.01 | [3] | 1119 |
| *trans,trans*-2,4-Octadienal[d, e] | 0.03 | [0] | 1125 |
| *trans*-2-Nonenal | 0.04 | [2] | 1167 |
| Myrtenal[d] | 0.28 | [1] | 1202 |
| Decanal[d, f] | 0.70 | [2] | 1207 |
| *trans,trans*-2,4-Nonadienal[d] | 0.22 | [3] | 1218 |
| *trans*-2-Decenal[d] | 0.02 | [2] | 1277 |
| Cinnamaldehyde[d] | tr | [1] | 1287 |
| Perillaldehyde[d] | 0.08 | [3] | 1288 |
| Undecanal[d] | tr | [1] | 1320 |
| *trans-trans*-2,4-Decadienal[d] | 0.03 | [1] | 1324 |
| *trans*,2-Undecanal | 0.06 | [2] | 1373 |
| Dodecanal[d] | 0.01 | [1] | 1413 |
| Tridecanal[d, e] | 0.17 | [2] | 1516 |
| Cyclamenaldehyde[e] | tr | [0] | |
| Tetradecanal[d] | 0.05 | [1] | 1617 |
| Pentadecanal[d] | 0.20 | [1] | 1712 |
| *Total percentage* | 5.12 | | |
| Ketones | | | |
| 3-Buten-2-one[e] | + | [0] | |
| 2,3-Butanedione | + | [2] | |
| 2-Butanone | + | [2] | |
| 3-Methyl-2-butanone | tr | [0] | |
| 2-Pentanone[d] | tr | [1] | |
| 3-Pentanone[d, f] | tr | [1] | |
| 2-Methyl-1-penten-3-one[f] | tr | [1] | |
| 3-Hydroxy-2-butanone[d] | tr | [1] | |
| 3-Penten-2-one[f] | 0.65 | [2] | |
| 4-Methyl-2-pentanone | tr | [1] | |
| 2-Heptanone[d, f, g] | 3.32 | [3] | 904 |
| 3-Octanone[d] | tr | [2] | 951 |
| 2,3-Octadienone | 0.24 | [1] | 988 |
| 6-Methyl-5-Hepten-2-one[d] | 0.18 | [2] | 990 |
| Pulegone[d] | 2.05 | [3] | 1012 |
| Acetophenone[d] | 0.22 | [2] | 1080 |
| *trans-trans*-3,5-Octadien-2-one[e] | 0.56 | [1] | 1085 |
| Camphor[d] | 0.27 | [2] | 1188 |
| 4-Methyl acetophenone[d] | 0.08 | [3] | 1190 |

**Table 1** (continued)

| Compounds | %[a] | [OI][b] | RI[c] |
|---|---|---|---|
| Verbenone[d] | 0.35 | [3] | 1218 |
| Carvone[d, g] | 1.14 | [3] | 1257 |
| Piperitone | tr | [1] | 1260 |
| Carvenone[e] | tr | [0] | |
| Isopiperitone[d] | 0.08 | [0] | 1285 |
| 2-Undecanone[d] | 0.66 | [1] | 1310 |
| Damascenone[d, f] | 0.02 | [1] | 1384 |
| 2-Dodecanone[d, f] | 0.23 | [3] | 1408 |
| Dihydro-$\beta$-ionone[d] | 0.04 | [2] | 1424 |
| $\beta$-Ionone[d] | 0.03 | [3] | 1500 |
| 2-Tridecanone[d] | 0.09 | [1] | 1509 |
| *trans*-Geranylacetone[e] | tr | [1] | |
| Propiovanillone | 0.07 | [0] | 1592 |
| 2-Oxacyclotetradecanone | 0.02 | [0] | 1765 |
| Hexahydro farnesyl acetone | tr | [0] | |
| 2-Oxacycloheptadecanone | tr | [0] | 1969 |
| *Total percentage* | 8.25 | | |
| *Acetals* | | | |
| 1,1'-Dimethoxy ethane[f, g] | tr | [0] | |
| 2,2'-Dimethoxy ethane | tr | [0] | |
| 1-Ethoxy-1-methoxy ethane | tr | [0] | |
| 1,1'-Dimethoxy propane | tr | [0] | |
| 1,1'-Diethoxy ethane[f, g] | tr | [1] | |
| 1,3'-Dimethoxy propane[e] | tr | [0] | |
| 1-Ethoxy-1-Propoxy ethane | tr | [0] | |
| 1,1'-Dimethoxy heptane | tr | [1] | |
| 1,1'-Dimethoxy octane[e] | tr | [2] | |
| 1,1'-Dimethoxy nonane | 0.01 | [1] | |
| 1,1'-Dimethoxy decane | 0.01 | [1] | |
| *Total percentage* | 0.02 | | |
| Phenols | | | |
| Eugenol[d, g] | 0.10 | [3] | 1361 |
| Methyl eugenol[d, f] | 0.02 | [2] | 1393 |
| Vanilline[d] | 0.05 | [3] | 1404 |
| Isoeugenol[d] | tr | [1] | 1450 |
| Methyl isoeugenol[f] | 0.02 | [3] | 1489 |
| 4-Allyl-2,4-Dimethoxy phenol | tr | [0] | |
| Elemicine[g] | 1.12 | [3] | 1566 |
| *Total percentage* | 1.31 | | |
| *Alcohols* | | | |
| Ethanol[d, g] | + | [0] | |
| 1-Penten-3-ol | tr | [0] | |
| 3-Methyl-1-butanol[d, f] | tr | [1] | |
| 1-Pentanol[d] | 0.67 | [3] | |
| 3-Methyl-2-buten-1-ol[e] | tr | [1] | |
| *cis*-3-Hexen-1-ol[d, f] | 0.43 | [1] | 868 |
| *trans*-2-Hexen-1-ol[d, f] | 0.14 | [1] | 875 |
| 1-Hexanol[d, f, g] | 3.05 | [2] | 879 |
| 2-Heptanol[d, f, g] | 43.06 | [3] | 914 |
| 1-Heptanol[d] | 0.06 | [3] | 978 |
| 2-Butoxy-ethanol[e] | tr | [0] | |
| 1-Octen-3-ol[d] | 0.45 | [3] | 984 |
| 6-Methyl-5-hepten-2-ol | 0.21 | [0] | 995 |
| 6-Methyl-1-heptanol | 0.26 | [1] | 1028 |
| 2-Ethyl-1-Hexanol[d] | 0.07 | [2] | 1039 |
| Phenylmethanol[d, f] | tr | [1] | 1046 |
| 1-Octanol[d, f] | 1.83 | [3] | 1086 |
| 2-Phenyl-2-propanol | 0.04 | [3] | 1104 |
| Linalool[d, f, g] | 0.96 | [3] | 1116 |
| Phenylethanol[d, f, g] | 0.21 | [2] | 1132 |
| *trans*-Pinocarveol[d, e] | 0.05 | [1] | 1146 |
| Isoborneol[d, f] | 1.76 | [3] | 1176 |
| 1-Nonanol[d] | 0.35 | [3] | 1179 |
| 4-Terpineol[d, g] | 1.21 | [2] | 1186 |
| *p*-Cymen-8-ol[d, f, g] | 3.72 | [3] | 1190 |
| $\alpha$-Terpineol[d, g] | 2.38 | [3] | 1195 |
| Myrtenol[d] | 1.28 | [1] | 1196 |
| *cis*-Piperitol[d] | tr | [1] | 1198 |
| Borneol[d, g] | 0.70 | [2] | 1207 |
| 3-Phenyl-1-propanol[d] | 0.02 | [1] | 1239 |
| Geraniol[d] | 0.19 | [3] | 1265 |
| 1-Decanol[d] | 0.28 | [3] | 1283 |
| *p*-1,4-Mentadien-7-ol | tr | [0] | |
| *p*-Cymen-7-ol[d, f] | 0.21 | [0] | 1307 |
| Perillyl alcohol[d] | 0.66 | [3] | 1312 |
| Thymol[d] | 0.61 | [2] | 1315 |
| *cis*-Cinnamic alcohol[d] | 0.07 | [3] | 1320 |

**Table 1** (continued)

| Compounds | %[a] | [OI][b] | RI[c] |
|---|---|---|---|
| *p*-1,5-Mentadien-7-ol | tr | [0] | |
| 1-Undecanol | tr | [1] | |
| 2-(3-isopropylphenyl)-1-propanol | tr | [0] | |
| 1-Decanol | 0.14 | [3] | 1481 |
| Cadinol | 0.21 | [0] | 1654 |
| *Total percentage* | 65.28 | | |
| *Lactones* | | | |
| γ-Butyrolactone[d, f] | 0.01 | [1] | 924 |
| γ-Hexalactone[d, f] | 0.20 | [3] | 1067 |
| γ-Octalactone[d] | 0.16 | [3] | 1270 |
| δ-Octalactone[d] | 0.16 | [3] | 1299 |
| γ-Nonalactone[d] | 0.07 | [3] | 1370 |
| γ-Decalactone[d] | 0.12 | [3] | 1480 |
| δ-Decalactone[d] | 0.03 | [2] | 1501 |
| γ-Undecalactone[d] | 0.06 | [0] | 1578 |
| δ-Undecalactone[d] | 0.04 | [1] | 1609 |
| γ-Dodecalactone[d] | 0.10 | [0] | 1690 |
| δ-Dodecalactone[d] | tr | [1] | 1703 |
| *Total percentage* | 0.95 | | |
| *Furans* | | | |
| Tetrahydrofuran[d, e] | tr | [0] | |
| 2-Methyl tetrahydrofuran | tr | [0] | |
| 3-Methyl tetrahydrofuran | tr | [0] | |
| 2-Methyl furan | tr | [0] | |
| 2,4-Dimethyl tetrahydrofuran[e] | tr | [0] | |
| 3-Methyl-(3H)dihydrofuran | 0.03 | [0] | 959 |
| 3,4-Dimethyl 2,5-furandione | tr | [0] | |
| Dibenzofuran[e] | tr | [0] | |
| *Total percentage* | 0.03 | | |
| *Ethers* | | | |
| 3-Methoxyhexane | 0.15 | [1] | 973 |
| 3-Methoxyoctane | 0.45 | [1] | 984 |
| 8-Ethoxy-*p*-cymene[e] | 0.80 | [2] | 1239 |
| *Total percentage* | | | |
| *Miscellaneous* | | | |
| 3,4-Dimethy-1,3-dioxane[e] | tr | [0] | |
| 2,4,5-Trimethyl-1,3-dioxolane[e] | tr | [0] | |
| Pyridine[e] | tr | [0] | |
| Methoxyfuraneol[d, f] | 0.07 | [2] | 1075 |
| 2-Methoxy-2-hydroxy-1-phenyl ethane | 0.06 | [0] | 1125 |
| Benzyl cyanide[e] | tr | [0] | |
| Benzothiazole | 0.01 | [1] | 1231 |
| Quinoline | tr | [2] | 1256 |
| *cis*-Theaspirane[e] | tr | [0] | 1321 |
| *trans*-Theaspirane | 0.02 | [0] | 1334 |
| Dimethylphenyl carbinol butanoate[e] | tr | [0] | |
| Dihydroactinidiolide | 0.01 | [3] | 1546 |
| *cis*-5,8-Megastigmadien-4-one[e] | tr | [0] | |
| Tonalide[e] | tr | [0] | |
| *Total percentage* | 0.17 | | |
| *Acids* | | | |
| Acetic acid[e] | + | [0] | |
| Hexanoic acid[e] | + | [0] | |
| Octanoic acid[e] | + | [0] | |
| Decanoic acid[e] | + | [0] | |
| Undecanoic acid[e] | + | [0] | |
| Dodecanoic acid[e] | + | [0] | |
| Tridecanoic acid[e] | + | [0] | |
| Tetradecanoic acid[e] | + | [0] | |
| Hexadecanoic acid[e] | + | [0] | |
| *Total percentage* | 86.17 | | |

[a] Relative amount in the dichloromethane fraction (i.e. hydrocarbons and acids excluded)

[b] Odour intensity: 0 no odour – 1 weak odour – 2 mediumly intense odour – 3 strong odour

[c] Retention indices determined on DB5 column

[d] Retention indices identical with those of authentic compounds

[e] Compounds for which EI mass spectra only have been obtained. In all other cases both EI and CI mass spectra were recorded

[f] Positively confirmed by infra-red spectrometry

[g] Compounds already identified in blackberry essence (1–3)

legone (2.05%), 1-octanol (1.83%), isoborneol (1.76%), myrtenol (1.28%), 4-terpineol (1.21%), carvone (1.14%), elemicine (1.12%), and nonanal (1.01%).

### *3.1 Alcohols*

Alcohols were the predominant chemical class mainly because of 2-heptanol which possessed intense fruity and herbaceous notes. Its odour has been described as reminiscent of blackcurrant leaves and artic bramble-like [7]. Terpenic alcohols found in blackberries have already been frequently identified in various fruits. Here they had intense flowery and fruity notes. *P*-cymen-8-ol, already mentioned in blackberry essence [2] was found again here. With its intense flowery and spicy odour, it was the second most abundant compound.

### *3.2 Ketones*

Ketones were the second most abundant chemical class. Among them, 2-heptanone, 4-methyl-acetophenone, verbenone, carvone, 2-dodecanone and β-ionone possessed the most intense and interesting odour.

According to Rogers and Toth [8], 4-methyl-acetophenone and carvone are produced by oxidation of terpinolene and limonene respectively during storage, whereas verbenone is produced from α-pinene [9].

### *3.3 Aldehydes*

Among the aldehydes, hexanal, heptanal, octanal, *trans*-2-octenal, nonanal, *trans-trans*-2,4-nonadienal and perillaldehyde seemed to contribute to the overall aroma of blackberry.

### *3.4 Esters*

Esters represented the more numerous compounds in blackberries but most of them were only present at traces levels. The most important ester was ethyl 2-methyl butanoate which had strong fruity and apple-like notes. We also identified ethyl 5-hydroxy hexanoate, ethyl 5-hydroxy octanoate and ethyl 5-hydroxy decanoate.

The last two compounds have already been mentioned as being present in wild and cultivated raspberries [10]. These compounds which were only present at trace levels were very unstable and their concentration decreased when the raspberries were deep-frozen and thawed or during isolation. Honkanen et al. [10] noticed that this degradation, probably due to both enzymatic and chemical reactions caused liberation of ethanol and possibly resulted in the formation of δ-lactones.

### 3.5 *Phenols*

Despite their low percentage occurrence, some phenols have a very important odour impact on blackberry, because of their low thresholds. The most important was elemicine (3,4,5-trimethoxyallyl benzene) with intense sweet flowery and woody notes. This compound has already been identified in blackberry essence [3], but it was not considered as important for the aroma. However, 3-dimethoxyallyl benzene, which imparted, according to Gulan et al. [3] an important musty note to the aroma, was not found in our extracts. Eugenol, isoeugenol and methyl-isoeugenol had very intense clove notes contributing with the same intensity to the overall profile. The sweet, vanilla-like odour of vanillin also seemed very important.

### 3.6 *Lactones*

The $\gamma$- and $\delta$-lactones between chain length of $C_6$ and $C_{10}$ seemed important for the background odour.

### 3.7 *Miscellaneous*

Two other compounds are worth mentioning: dihydroactinidiolide and methoxyfuraneol [2,5-dimethyl-4-methoxy-3(2H)furanone]. The former contributed positively to the aroma of blackberry with its intense fruity odour and has been reported as a product of carotenoids degradation [11, 12]. The latter, known also as "mesifuran" [13], imparted sweet cherry-like and herbal notes to the blackberry aroma.

The acetals found here had rather weak odours and the amounts of furans were so low that no odour could be perceived at the sniffing-port of the chromatograph. Both classes can be considered as artefacts, possibly produced during isolation or concentration.

### 3.8 *Unidentified compounds*

A few compounds, which seemed important to blackberry aroma because of their noticeable odour or their abundance, remained unidentified. Mass spectral data of the most important compounds are presented (Table 2). EI-MS and IR spectra are presented for four of them.

The unknown compound No. 5 which had a molecular weight of 168 and an intense fruity odour seemed, from its IR spectrum, to be a keto-alcohol. A non-identified compound exhibiting the same molecular weight and a strong blackberry-like odour was already mentioned in blackberry [3]. Unfortunately, as Gulan et al. [3] did not give any spectral information, it is impossible to know if these two compounds are identical.

Compound No. 14 seemed to be an ester as its IR spectrum showed bands at 1735 $cm^{-1}$ and 1259 $cm^{-1}$ which characterised C=O and C–O functions respectively.

The unknown compound No. 18 which eluted in the fraction No. 7 (pentane: dichloromethane, 70:30) had an intense flowery and spicy odour. Its molecular weight 180 was confirmed by CI-MS. The absorption band at 1798 $cm^{-1}$ in its IR spectrum was characteristic of the $\nu$(C=O) vibration of a $\gamma$-lactone. This band usually found at 1810 $cm^{-1}$ was displaced because of the conjugated double bond (the presence of which

**Table 2.** Non identified compounds of fresh blackberries

| Peak no. | EI-MS data (m/z [%])[a] | Presumed chemical class | Odour description | %[b] [OI][c] | RI[d] | MW[e] |
|---|---|---|---|---|---|---|
| 1 | 43(100), 41(35), 71(34), 93(21), 81(19), 111(17), 55(16), 39(14) | Terpenic alcohol | Flowery | 1.00 [2] | 1115 | 154 |
| 2 | 43(100), 41(41), 55(20), 93(19), 69(18), 139(16), 71(12), 81(11) | Terpenic alcohol | Flowery | 0.08 [2] | 1139 | 154 |
| 3 | 41(100), 55(60), 92(52), 43(42), 70(41), 83(33), 91(32), 93(30) | | Flowery | 0.16 [3] | 1153 | 150 |
| 4 | 83(100), 41(62), 55(59), 95(47), 39(38), 109(26), 67(21), 43(17) | Terpenic alcohol | Fruity-etheral | 0.11 [3] | 1211 | 152 |
| 5 | 43(100), 41(40), 95(34), 97(26), 55(21), 39(19), 83(19), 82(18) | Insaturated ketonic alcohol | Fruity | 1.03 [2] | 1216 | 168 |
| 6 | 43(100), 119(46), 59(40), 41(28), 136(17), 55(16), 95(14), 91(13) | Terpenic alcohol | Flowery | 0.10 [3] | 1235 | 152 |
| 7 | 43(100), 93(46), 41(44), 39(23), 79(19), 91(16), 94(13), 119(14) | Terpenic alcohol | Fruity | 0.12 [3] | 1249 | 152 |
| 8 | 121(100), 91(67), 94(64), 77(53), 39(52), 150(45), 41(44), 93(38) | Oxygenated terpenoid | Musty | 0.03 [2] | 1330 | 150 |
| 9 | 121(100), 91(74), 79(72), 41(70), 93(65), 39(55), 43(54), 134(44) | Terpenic alcohol | Fruity | 0.18 [2] | 1348 | 152 |
| 10 | 43(100), 84(63), 55(54), 41(46), 125(23), 39(19), 83(10), 99(10) | Oxygenated terpenoid | Fruity | tr [2] | 1357 | 154 |
| 11 | 85(100), 105(58), 77(30), 134(24), 41(24), 73(16), 51(12), 39(11) | | Fruity | tr [3] | | 150 |
| 12 | 43(100), 121(85), 93(73), 136(31), 91(29), 41(27), 77(13), 169(10) | Terpene | Flowery | 0.04 [3] | 1427 | 136 |
| 13 | 123(100), 41(60), 43(56), 81(47), 95(38), 163(32), 45(32), 93(29) | Alcohol | Sweet | 0.03 [2] | 1458 | 196 |
| 14 | 55(100), 41(36), 84(35), 43(27), 111(20), 129(20), 42(20), 56(20) | Ester | Fruity | 0.06 [3] | 1494 | 182 |
| 15 | 141(100), 41(19), 43(11), 113(9), 55(7), 142(7), 39(6), 54(6) | | Flowery | 0.17 [2] | 1513 | 212 |
| 16 | 135(100), 41(60), 96(44), 43(38), 55(33), 124(31), 81(29), 95(24) | | Musty | 0.02 [3] | 1524 | |
| 17 | 43(100), 41(44), 207(37), 161(30), 105(29), 55(23), 91(20), 121(6) | Sesquiterpenic alcohol | Floral | 0.07 [2] | 1529 | 222 |
| 18 | 55(100), 124(91), 137(66), 41(34), 39(25), 125(25), 54(22), 180(21) | | Flowery fruity | 0.23 [3] | 1534 | 180 |
| 19 | 221(100), 161(77), 41(62), 41(54), 105(50), 85(46), 91(43), 93(38) | Sesquiterpenic alcohol | Flowery | 0.10 [2] | 1546 | 222 |
| 20 | 41(100), 93(57), 43(53), 107(50), 55(48), 91(45), 79(39), 95(35) | Oxygenated sesquiterpenoid | Flowery | 0.20 [3] | 1628 | 220 |

[a] Relative intensities, with the base peak taken at 100, are given in parentheses
[b] Relative amount in the dichloromethane fraction
[c] Odour intensity
[d] Retention indices calculated on DB5 column
[e] Molecular weight (verified by chemical ionization mass spectrometry)

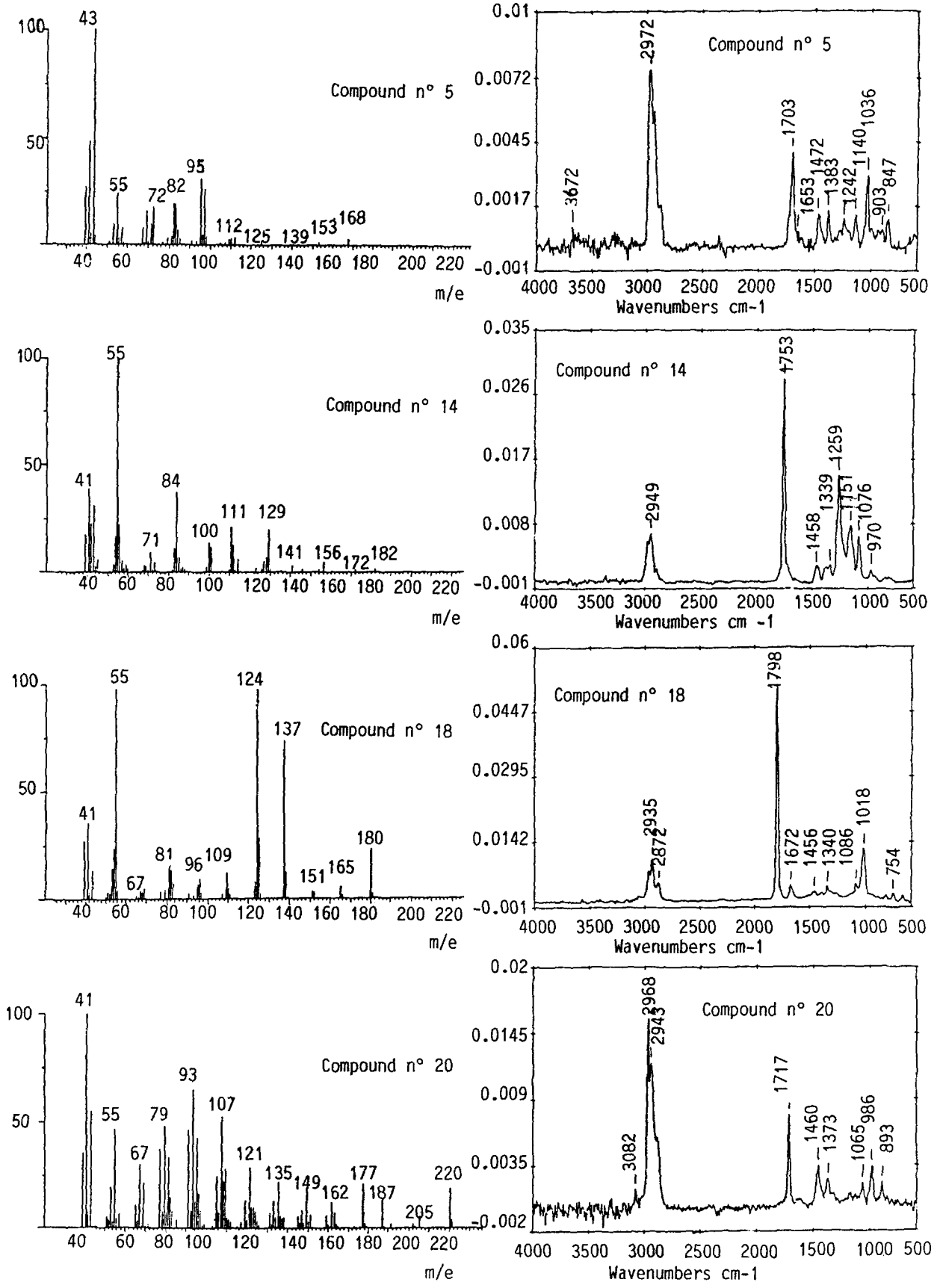


**Fig. 1.** Mass and infra-red (vapour phase) spectra of unidentified compounds No. 5, 14, 18, and 20

was suggested by the 1672 $cm^{-1}$ absorption band). Two formulae can be proposed: $C_{12}H_{20}O$ and $C_{11}H_{16}O_2$ with a total number of rings and double bonds of 3 and 4 respectively. Both MS and IR data are very similar to those of bovolide (2,3-dimethyl-4-hydroxy-2,4-nonadienoic acid $\gamma$-lactone) [14], which contains one ring and three double bonds but a reference vapour phase IR spectrum would be useful, in order to confirm this conclusion.

Compound No. 20 seemed to be an oxygenated sesquiterpene (molecular weight 220) probably a terpenic alcohol because of the presence of a M-17 fragment in the CI-MS. According its EI-MS spectrum, this compound probably derived from aromadendrene and could be an isomer of spathulenol.

## 4 Conclusions

Despite the large number of volatiles positively identified, no single compound identified or remaining unknown could be described as blackberry-like. Different attempts to reproduce the original aroma by mixing adequate amounts of pure compounds chosen from among the most abundant, resulted in an odour somewhat reminiscent of blackberries, but lacking the delicate aroma of the natural extract. So the blackberry aroma is a complex mixture in which minor compounds may not be neglected as they are of great importance for the balance and the refinement of the overall profile.

*Acknowledgement.* We are grateful to Mr. Aravantinos (I.F.F., France) for supplying the blackberries.

## References

1. Scanlan RA, Bills DD, Libbey LM (1970) J Agric Food Chem 18:744
2. Houchen M, Scanlan RA, Libbey LM, Bills DD (1972) J Agric Food Chem 20:170
3. Gulan MP, Veek MH, Scanlan RA, Libbey LM (1973) J Agric Food Chem 21:741
4. Guichard E, Issanchou S (1983) Sci Alim 3:427–438
5. Loyaux D, Roger S, Adda J (1981) J Sci Food Agric 32:1254–1258
6. Palmer JK (1973) J Agric Food Chem 21:923–925
7. Pyysalo T, Suihko M, Honkanen E (1977) Lebens Wiss Technol 10:36–39
8. Rogers J, Toth Z (1968) Cereal Sci Today 13:294
9. Banthorpe DV, Charlwood BV, Francis JO (1972) Chem Rev 72:115
10. Honkanen E, Pyysalo T, Hirvi T (1980) Z Lebensm Unters Forsch 171:180–182
11. Isoe S, Hyeon SB, Sakan T (1969) Tetrahedron Lett 4:279
12. Etoh H, Ina K (1979) Agric Biol Chem 43:2593
13. Kallio H (1976) J Food Sci 41:555–562
14. Horita H, Hara T, Sannai A, Fujimori T (1985) Agric Biol Chem 49:3601–3603

Received September 12, 1986

Accepted October 10, 1986