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Volatile compounds from leaf-buds of *Populus nigra* L. (Salicaceae)

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Abstract

Volatile components from fresh and air-dried leaf-buds of *Populus nigra* L. (Salicaceae) were isolated by Likens-Nickerson apparatus and analyzed using GC/MS. Forty-eight components (ca. 95% of the total isolate) were identified among black poplar bud volatiles. Sesquiterpene alcohols β-eudesmol and α-eudesmol represented 26.3–28.7% of the oil. Other major sesquiterpene compounds were γ-selinene (7.6–8.8%), δ-cadinene (7.8–8.6%), α-elemene (3.3–5.2%) and γ-cadinene (3.9–4.2%). Hemiterpenes were also identified (2.2–7.6%). Monoterpenes were present in low percentages (1.6–5.7%). Aliphatic and aromatic alcohols, carbonyl compounds and aliphatic acids were identified among non-terpene volatiles (9.8–13.5%). The fresh buds contained 0.27% and dried 0.12% essential oil. Air-drying moderately effected the volatiles qualitative and quantitative composition.

Keywords: *Populus nigra* L.; *Gemmae populi*; Salicaceae; Black poplar; Volatiles; GC/MS

1. Introduction

Populus nigra L., commonly known as black poplar, is a member of the Salicaceae family. It is a deciduous tree with broad green leaves and small yellowish-green flowers. Black poplar grows in southern and central Europe, central Asia, Siberia, North America and it can also be found in Croatia. It thrives in the light, rich vegetation that may be found near rivers. There are two broadly accepted groupings, *P. nigra* var. *betulifolia* Torrey, the English or pubescent black poplar, and *P. nigra* var. *typica* Schneider, the continental or glabrous black poplar (Elwes and Henry, 1913; Arbez and Lefèvre, 1997; Turok et al., 1998). Poplar bark is tonic and febrifuge. As a diuretic it has been beneficially used in curing urinary affections. The glucosides salicin and populin are constituents common to the barks of nearly all species of *Populus* as well as different willows, especially *Salix alba* L.

The leaf-buds of black poplar (*Gemmae populi*) are used in traditional medicine as well as their alcoholic extracts. The constituents of alcoholic extracts and propolis have been compared and shown to be similar in many respects (Nagy et al., 1986). The bud exudates of *P. nigra* from different origin contain caffeic and isoferulic acids with their esters, chalcones, flavanones and flavones as the major components (Greenaway et al., 1990a,b). Later, Greenaway et al. (1988) identified novel isoferulate esters in the bud exudates of *P. nigra*. The composition of bud and leaf exudates of some *Populus* species were also compared (Greenaway et al., 1992a,b). In recent time, the leaf-buds exudate of *P. nigra* and propolis from Poland were found to contain different flavaonoids (Macijewicz et al., 2001). Further, black poplar buds contain about 0.5% volatile oil (Gildmeister and Hoffmann, 1956), but the data on its chemical composition is limited.

The bud volatiles of black poplar are in the focus of this research. The samples of essential oil from a simultaneous hydrodistillation-extraction (Lickens–Nickerson apparatus) have been analyzed by a gas chromatography/mass spectrometry (GC/MS). For this investigation we used fresh and air-dried leaf-buds. A detailed research of the bud volatiles from this tree has not yet been undertaken.

2. Results and discussion

The simultaneous hydrodistillation-extraction (Lickens-Nickerson apparatus) of *P. nigra* leaf-buds yielded a

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clear and colourless oil. The oil yield from fresh buds was 0.27% (of fresh wt.). A significant reduction in the volatiles amount was found in the dried buds (0.12% of fresh wt.). Whatley et al. (1989) found that *P. nigra* buds exudate contained 0.5% terpene compounds. Further studies of Greenaway et al. (1990a) on the variations of *P. nigra* buds exudate composition showed the absence of terpene compounds from 12 poplar exudates originated from seven countries or they comprised $<0.1\%$ of the exudates. Identified terpenes were not isolated as the volatiles but were noted among minor compounds of the bud exudates and extracts.

To detect the overlapping compounds (as well as those with small concentration), the isolated oil was fractionated to obtain the fractions of hydrocarbons and oxygen-containing compounds. The oil and fractions were analyzed on two columns with different stationary phase polarity (HP-20M and HP-101). It is obvious, from the analysis of oil and its fractions, that the fractionation contributes to qualitative and quantitative oil analysis. In total, 48 compounds representing 93.5–94.4% of the overall black poplar volatiles were identified on the basis of mass spectra and retention indices. Unidentified components (ca. 5%) were present in such low amounts that either no mass spectrum could be recorded or the spectrum was too poor for interpretation. The fully identified components are reported as black poplar bud volatiles for the first time.

The identified components and their percentages are given in Table 1, where the components are grouped by chemical classes and listed in order of their elution from HP-20M column within each class. An immediate observation was the presence of high level of sesquiterpenes, although with remarkable differences concerning the main components. Sesquiterpene compounds are widespread but usually minor components of plant oils, but often endow crucial flavour characteristics (Banthrope, 1996). Sesquiterpene alcohols β-eudesmol and α-eudesmol presented as much as 26.3-28.7% of the total isolate (Table 1). α-Eudesmol was differentiated from β-eudesmol not only by retention indices, but also by mass spectra (Adams, 1995). There are publications which reported the detection of α/β-eudesmols from different essential oils (Lis and Gora, 2001; Pino et al., 2002). Eudesmols are derived from cyclization of hedycaryol during plant ageing or during the process of oil extraction by distillation (Cornwell et al., 2000). High percentage of eudesmols in the isolate could be of interest since Miyazawa et al. (1996) investigated the antimutagenic activity of β-eudesmol and paeonol from *Dioscorea japonica*. α-Copaene-11-ol (1.4–5.4%), δ-cadinol (3.0–3.6%), torreyol (2.0–2.4%) and elemol (0.4–0.5%) were also identified among sesquiterpene alcohols. Elemol is a thermal Cope rearrangement product of hedycaryol (Chamblee et al., 1997; Cornwell et al., 1999) formed either in the injector of a gas chromatograph, during a distillation or during leaf-buds development. The presence of eudesmols and elemol can be taken as the evidence of hedycaryol occurence in the buds, although it was not detected in the oil, Table 1. Three unidentified sesquiterpenols were previously detected in the bud exudate of *P. trichocarpa* and *P. generosa* (Greenaway et al., 1990b). Nerolidol and bisabolol were also identified in *P. generosa*. Bisabolol was present with 16.1% in the bud extract of *P. candicans* (Whatley et al., 1989) while it was not identified in our samples.

The important representatives of sesquiterpene hydrocarbons were γ-selinene (7.6–8.8%), δ-cadinene (7.8–8.6%), α-elemene (3.3–5.2%) and γ-cadinene (3.9–4.2%), Table 1. Other sesquiterpene hydrocarbons present in small quantities were α-muurolene (2.0–2.5%), α-humulene (1.9–2.3%), alloaromadendrene (0.8–1.0%), and β-caryophyllene (0.1–0.3%).

The rather small monoterpene fraction, ranging up to 5.7% of the total bud volatiles, was dominated by oxygen-containing monoterpenes eugenol (1.1–3.9%), methyl eugenol (0.3–0.5%), linalool (0.2–0.4%) with minor percentage of geraniol and β-cyclocitral, Table 1.

Aliphatic alcohols 2-methyl-3-buten-2-ol, 3-methyl-1-butanol, (*E*)-2-methyl-2-buten-1-ol and (*E*)-2-metyl-2-butenoic acid belong to hemiterpene compounds derived from 3,3-dimethylallyl pyrophosphate and isopentenyl pyrophosphate (Banthrope, 1996). Hemiterpenes were present in total amount $<8\%$. The esters of hemiterpene (prenyl) alcohols and *cis*/*trans* caffeic, ferulic and isoferulic acids were previously identified in the bud exudate of *P. nigra* (Greenaway et al., 1988, 1990a). They are non-volatile compounds and can not be isolated by the simultaneous hydrodistillation-extraction. It is interesting to note that acetate of 3,3-dimethylalyl alcohol is bee alarm pheromone (Banthrope, 1996). The prenyl esters are of particular interest because 3-methyl-2-butenyl caffeate has been found to be the major contact allergen of the bud exudates of *P. nigra* (Hausen et al., 1987a,b).

Non-terpenes presented 9.8–13.5% of the total isolate and the gross composition of non-terpenes were aliphatic and aromatic alcohols, carbonyl compounds and aliphatic acids. The main representatives of aromatic non-terpene alcohols were 2-phenylethanol (1.1–2.0%), 3-phenyl-2-propen-1-ol (0–1.1%), 1-phenyl-2-propen-1-ol (0.2–0.8%), and benzyl alcohol (0.6–0.8%). Benzyl alcohol and 2-phenylethanol were previously identified (ca. 0.2%) in the bud extract of *P. candicans* (Whatley et al., 1989). Identified aliphatic and aromatic aldehydes and ketones were 1,2-cyclohexanedione (1.0–2.6%), 1-phenylethanone (0.2–1.0%), benzaldehyde (0.2–0.5%) with its 2-hydroxy derivative, salicyl aldehyde

Table 1
Volatiles from leaf-buds of *Populus nigra* L.

No.	Compound	RI (HP-20M)	Peak area (%)	
			Fresh buds	Dried buds
Hemiterpenes				
1.	2-Methyl-3-buten-2-ol	1035	0.6	2.3
2.	3-Methyl-1-butanol	1185	–	1.2
3.	(*E*)-2-Methyl-2-buten-1-ol	1292	0.9	3.5
4.	(*E*)-2-Methyl-2-butenoic acid	1792	0.7	0.6
Monoterpenes				
5.	Linalool	1519	0.2	0.4
6.	β-Cyclocitral	1585	–	0.4
7.	Geraniol	1806	–	0.5
8.	Methyl eugenol	1982	0.3	0.5
9.	Eugenol	2114	1.1	3.9
Sesquiterpene hydrocarbons				
10.	α-Cubebene	1446	–	0.3
11.	α-Copaene	1478	1.0	0.8
12.	β-Caryophyllene	1575	0.3	0.1
13.	Alloaromadendrene	1616	1.0	0.8
14.	α-Humulene	1643	1.9	2.3
15.	Epizonaren	1688	1.7	2.2
16.	α-Muurolene	1697	2.5	2.0
17.	γ-Cadinene	1699	3.9	4.2
18.	δ-Cadinene	1743	7.8	8.6
19.	1,2,3,4-Tetrahydro-1,6-dimethyl-4-isopropylnaphtalene	1805	1.6	2.2
20.	γ-Selinene	2138	7.6	8.8
21.	α-Elemene	2150	5.2	3.3
Sesquiterpene alcohols				
22.	α-Copaene-11-ol	1999	5.4	1.4
23.	Elemol	2036	0.4	0.5
24.	T-Cadinol	2132	1.2	2.5
25.	Torreyol	2147	2.4	2.0
26.	δ-Cadinol	2156	3.6	3.0
27.	β-Eudesmol	2189	19.1	19.6
28.	α-Eudesmol	2230	9.6	6.7
Other compounds				
29.	Benzaldehyde	1433	0.2	0.5
30.	2-Methylpropanoic acid	1527	0.1	–
31.	1,2-Cyclohexanedione	1593	2.6	1.0
32.	1-Phenylethanone	1608	1.0	0.2
33.	2-Methylhexanoic acid	1625	0.9	0.4
34.	Salicyl aldehyde	1633	0.2	1.4
35.	Benzyl alcohol	1825	0.6	0.8
36.	2-*H*-Isoindole	1852	0.8	–
37.	2-Phenylethanol	1863	1.1	2.0
38.	1,2-Dihydro-1,1,6-trimethylnaphtalene	1884	0.8	1.0
39.	1-Phenyl-2-propen-1-ol	1918	0.8	0.2
40.	3-Menthyl-2-phenylethyl butanoic acid	1934	0.3	0.2
41.	Phenol	1945	0.7	0.6
42.	Octanoic acid	2005	0.6	0.2
43.	4-Phenyl-3-buten-2-one[a]	2058	0.2	0.3
44.	3-Phenyl-2-propen-1-ol acetate[a]	2104	0.6	–
45.	Decanoic acid	2235	–	0.1
46.	3-Phenyl-2-propen-1-ol[a]	2252	1.1	–
47.	Docosane	2391	0.7	0.9
48.	Dodecanoic acid	2677	0.2	–
Total of grouped compounds (%)				
Hemiterpenes			2.2	7.6
Monoterpenes			1.6	5.7
Sesquiterpene hydrocarbons			35.2	36.5
Sesquiterpene alcohols			41.7	35.7
Other compounds			13.5	9.8
Total identified			93.5%	94.4%
Yield (of fresh wt.)			0.27%	0.12%

RI, retention indices on HP-20M column.
[a] Correct isomer is not identified.

(0.2–1.4%) and 4-phenyl-3-buten-2-one (0.2–0.3%). In this group of the poplar buds non-terpenes aliphatic acids with 8, 10 and 12 carbon atoms were identified, as well as 2-methyl derivatives of hexanoic and 2-butenoic acid and 3-methyl-2-phenylethylbutanoic acid.

Although the constituents of poplar buds alcoholic extracts and propolis have been compared and shown to be similar among non-volatile compounds (Nagy et al., 1986), the volatiles of Croatian propolis (Borčić et al., 1996) and black poplar buds strongly differ.

The reduction in amounts of volatiles during drying process depends on the volatility (physicochemical properties) and botanical structure of the particles that store the essential oil. Although the leaf-buds contain a lot of nonpolar compounds (waxes and lipids) a significant loss of volatiles was noted in dried leaf-buds (ca. 2 times). Air-drying moderately effected the poplar bud volatiles qualitative and quantitative composition. It is well known that drying of the plant material could also have an effect on glycosides (Trim, 1955), since the enzymes are still active for some time after plant collection and could hydrolyzed glucosides such as salicin and populin, found in black poplar (Greenaway et al., 1992a,b). The GC/MS analysis showed the absence of corresponding volatile aglycone (salicyl alcohol) after drying. However, we identified salicyl aldehyde in both materials in fresh 0.2% and in dried 1.4%. This compound may originate from salicin and/or populin by hydrolysis and oxidation. The prenyl alcohols can be liberated, during drying, by hydrolysis of non-volatile *cis*/*trans*- caffeic, ferulic and isoferulic prenyl esters. Namely, in our sample, prenyl alcohols were identified with higher percentage (7.6%) in dried buds, Table 1.

Finally we concluded from these results that qualitative composition of black poplar buds volatiles strongly differs from the composition of terpenoids found in the bud excudates and extracts from *Poplar* species (Whatley et al., 1989; Greenaway et al., 1990b). These differences seems to be mostly due to applied method of isolation since in previous researches non-volatile compounds from the bud exudates and extracts were isolated and analysed. The isolated essential oil of *P. nigra* leaf-buds mainly contained sesquiterpene alcohols α-eudesmol and β-eudesmol. Other abundant sesquiterpene compounds were γ-selinene, δ-cadinene, α-elemene and γ-cadinene. Minor important constituents such as hemiterpenes, monoterpenes and other compounds were also identified. These compounds may be considered as common among black poplar buds volatiles. The present study reports results from a local variety originated from south Croatia-Dalmatia and in fact further investigations of *P. nigra* volatiles might supplement and broaden the knowledge of black poplar phytochemistry, due to limited data on *Poplar* volatile compounds.

3. Experimental

3.1. Plant material

Populus nigra L. leaf-buds were collected from the wild growing trees near Sinj, south Croatia in March 2002. These trees were considered to be typical of *P. nigra* at the original locality, and not hybrids. Half of the collected leaf-buds were immediately investigated and the other part was dried in a shaded place at room temperature for 15 days and then analysed as dried buds. The voucher specimens (No. 0011010-14) are deposited at the Department of Organic Chemistry, Faculty of Chemical Technology, Split.

3.2. Isolation of volatiles

The essential oil was isolated by simultaneous hydrodistillation-extraction (Likens and Nickerson, 1964) for 3 h with solvent penthane/ether (1:1 v/v). Pentane/ether extract was dried over anhydrous sodium sulphate. The solvent was removed by fractional distillation. The applied chemicals were purchased from Fluka Chemie, Buchs, Switzerland.

3.3. Fractionation of the essential oil

The isolated oil (20 μl) was fractionated on a silica gel microcolumn (30–60 μm, 500 mg, Merck, Darmstadt, Germany) and two fractions were obtained. Pentane (10 ml) is used for elution of nonpolar hydrocarbons and ether (10 ml) for elution of polar oxygen containing compounds. The applied solvents were purchased from Fluka Chemie, Buchs, Switzerland. The both fractions were concentrated to 0.5 ml and tested by thin layer chromatography. The first fraction contained only nonpolar hydrocarbons. The second fraction contained oxygenated terpenes and other polar compounds.

3.4. Gas chromatograpy/mass spectrometry (GC/MS)

Both fractions of the essential oil as well as the overall oil were analysed by gas chromatography/mass spectrometry (Hewlett Packard, Vienna, Austria, model 5890 series II with mass selective detector, model 5971A). Two columns of different polarity were used: a HP-20M column (Carbowax 20M, Hewlett Packard, Vienna, Austria; 50 m×0.2 mm i.d., film thickness 0.2 μm) and a HP-101 column (dimethylpolysiloxane fluid, Hewlett Packard, Vienna, Austria; 25 m×0.2 mm i.d., film thickness 0.2 μm). GC operating conditions were similar as in our previous papers (Jerković et al., 2001; Mastelić and Jerković, 2002). Oven temperature was programmed as follows: isothermal at 70 °C for 4 min, then increased to 180 °C, at a rate of 4 °C min^{-1} and subsequently held isothermal for 15 min (for HP-20M

column); isothermal at 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C min^{-1} and held isothermal for 15 min (for HP-101 column). Carrier gas was helium, flow rate: 1 ml min^{-1}; injector temperature: 250 °C ; volume injected: 1 μl; split ratio: 1:50. MS conditions: ionisation voltage: 70 eV; ion source temperature: 280 °C; mass range: 30–300 mass units.

3.5. Qualitative and quantitative determination

Duplicate analyses were performed. Quantitative results are mean of data derived from GC–MS analyses on two columns. The individual peaks were identified by comparison of their retention indices to those of authentic samples, as well as by comparing their mass spectra with the Wiley library mass spectral database and literature (Adams, 1995). Chirality analysis was not performed. The percentage composition of the samples was computed from the GC peak areas. For quantification of the oil fractions, internal standard (menthol, Fluka Chemie, Buchs, Switzerland) was added. Preliminary GC/MS analysis showed the absence of menthol among the buds volatiles. The oil yield was determined by gravimetric method and calculated as percentage respecting the mass of starting fresh plant material. This mode of representing the results enables the determination of drying impact on the volatiles content and composition.

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