GC-MS Analysis of Compounds Extracted from Buds of *Populus balsamifera* and *Populus nigra*

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The composition of hexane and ether extracts from buds of two poplar species (*Populus balsamifera* and *P. nigra*) was investigated by GC-MS method. In hexane extracts, 54 "neutral" compounds were recorded. The greatest amounts of them are sesquiterpenes and *n*-alkanes. Among 56 components of ether extracts, many aliphatic acids and hydroxyacids were detected. However, the main fraction consists of phenolcarboxylic acids, substituted cinnamic acids, and their esters. It was established that chemotaxonomic differences between *Populus balsamifera* and *P. nigra* are observed in the case of both hexane and ether bud extracts.

Key words: Populus balsamifera, *Populus nigra*, Bud Extracts, GC-MS Analysis

Introduction

The exudate from buds of many plant species of the *Populus* genus is the principal initial component of the plant material processed by bees into propolis (Marcucci, 1995). This valuable product has long been widely used in popular medicine for treating wounds and ulcers. The antiseptic properties of poplar buds exudate and propolis are mainly due to phenol carboxylic acids (PCA) and flavonoids contained in them. The same components principally determine poplar resistance to microbial infection (Scaysbrook *et al.*, 1992).

The composition of bud exudate is characteristic of different species or even clones of poplar (Greenaway *et al.*, 1989). Therefore, its investigation is of interest from the viewpoint of chemotaxonomy of this polymorphous genus of the *Salicaceae* family and its selection. The main characteristic used in the chemosystematics of poplar is the composition of phenol compounds: PCA, their esters and flavonoids. Hence, in recent years, many investigations dealt with the phenolic (acidic in nature) component of buds exudates of European, Asiatic, and North American poplar species (Scaysbrook *et al.*, 1992; Greenaway and Whatley, 1991; Greenaway *et al.*, 1992a, 1992b; English *et al.*, 1992; Maciejewicz *et al.*, 2002).

"Neutral" exudate components, such as terpenoids, have been much less investigated. These compounds are also widely represented in the plant world, their biosynthesis is different for each species, and therefore, their composition may be used for chemosystematics. However, the methodology used in the preparation of exudates for analysis (extraction by a polar organic solvent, its complete distillation, and lyophilic drying before derivatization) (Greenaway *et al.*, 1992a, 1992b; English *et al.*, 1992; Maciejewicz *et al.*, 2002) lead to a loss of a considerable part of relatively volatile terpenoids.

In the present paper we report the results of simultaneous determination of "neutral" and "acidic" compounds extracted by solvents of different polarities from buds of two poplar species: *Populus balsamifera* L. (section Tacamachaca) and *Populus nigra* var. *pyramidalis* (section Aigeiros).

Experimental

Sample preparation and analysis

Buds of two poplar species were collected in April 2002 from trees growing in the Białystok park zone. Freshly collected buds (5 g) were placed in vessels with sealed stoppers, 25 ml of *n*-hexane was poured on them, and the mixture was kept for 4 h with periodic stirring. After this the solvent was poured off, the buds were washed with 15 ml of *n*-hexane, and the solvent was removed from the combined extracts to a volume of 0.5 ml under vacuum on a rotor evaporator.

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After extraction with hexane, the buds were dried in air and crushed. To the resulting powder was added 25 ml of diethyl ether and mixture was stored for 4 h. The ether extracts were filtered; the residue was washed with 10 ml of ether and evaporated to dryness under vacuum. To the dry residue 50 μl of pyridine and 100 μl of BSTFA (Sigma) were added. The resulting solution was heated for 1 h at 60 °C to form trimethylsilyl derivatives (TMS).

Hexane extracts and solutions of TMS derivatives were analyzed by GC-MS on a Perkin-Elmer Turbo-Mass instrument supplied with a PE-5HT fused silica capillary column (30 m × 0.25 mm). Helium flow rate through the column was 1 ml min^{-1} with a 1:50 split. The injector temperature was 250 °C. Hexane solutions were separated in the temperature programming regime from 40 to 280 °C at a rate 3 °C min^{-1}. The initial column temperature for the separation of TMS was 50 °C, a temperature rise to 250 °C was accomplished at a rate of 5 °C min^{-1}.

A mixture of C_8–C_{31} *n*-alkanes was previously separated under the above conditions, and their retention times were determined. Linear retention indices were calculated from the results of the chromatography of these mixtures and extracts, and after integration the fraction of each component in the total ion current (TIC) was calculated.

Identification of components

To identify the mixture components, both mass spectral data and calculated retention indices (RI^{exp}) were used. Mass spectrometric identification was carried out with the aid of an automated system, which formed a part of the instrumentation used. Identification consists in the comparison of mass spectra recorded during the analysis and those contained in the instrument library.

Retention indices of "neutral" components of hexane extracts were compared with those reported by Adams (1995). The RI values for TMS were determined from the analysis of derivatives of authentic commercial preparations or taken from different papers (Tanaka and Hine, 1982; Tuchman *et al.*, 1984; Lefevere *et al.*, 1989; Greenaway and Whatley, 1991; Greenaway *et al.*, 1992a, 1992b; English *et al.*, 1992). When not less than three RI values are given for the same compound in different sources they were randomized.

Results and Discussion

Table I lists about 50 "neutral" compounds present in hexane extracts from buds of two poplar species in amounts of not less than 0.1% of TIC. There was some uncertainty when the literary values of retention indices were absent or when the differences between RI^{exp} and RI^{lit} were considerable (more than 5 index units). In these cases the component name is followed by a question mark indicating that its identification is not unambiguous.

The components listed in Table I can be divided into several groups the contribution of which to TIC are different for *P. balsamifera* and *P. nigra.* The first group (retention index range 1030–1360) consists of aromatic compounds: benzyl alcohol, 2-phenylethanol, eugenol, 2-hydroxybenzaldehyde, methyl acetophenone, and ethyl benzoate. Their fraction in extractive "neutral" compounds is not great: 3.0 and 5.7% TIC for *P. balsamifera* and *P. nigra*, respectively.

The second group is formed by sesquiterpene hydrocarbons (RI range 1400–1500) and sesquiterpenoids (RI range 1530–1670). In extracts from *P. balsamifera* buds, terpenoids fraction was 37.4%. It was reported (Greenaway *et al.*, 1992b) that exudates of *P. nigra* buds virtually do not contain terpenoids and greatly differ in this characteristic from *P. balsamifera.* Terpenoids are also absent from the list of compounds identified in the *P. nigra* exudates by Maciejewicz *et al.* (2002). However, according to our data, terpenoids group is second in importance among the components of hexane extracts from buds of this poplar species. The discrepancy is probably due to specific exudate samples preparation (Greenaway *et al.*, 1992a, 1992b; English *et al.*, 1992; Maciejewicz *et al.*, 2002) which we have mentioned in the introduction. Their methodology involves at least partial loss of relatively volatile compounds. The actual difference between terpenoids composition in bud extracts of these two poplar species is the almost complete absence of sesquiterpene $C_{15}H_{24}$ hydrocarbons in *P. nigra.*

Among sesquiterpenoids in buds of both poplar species, tertiary bicyclic alcohols with a structure of azulene type (guaiol and bulnesol) are prevailed. In the case of *P. balsamifera*, the contribution of isomeric alcohols of the selinane series (α-, β- and γ-eudesmol) is also considerable (9%).

Table I. Composition of "neutral" compounds in hexane extracts from poplar buds.

Compound	RI^{exp}	RI^{Lit} (Adams, 1995)	Content, rel. %	
			P. balsamifera	*P. nigra*
Heptanal	892	899	0.1	–
1,8-Cineole	1023	1022	–	0.8
Benzyl alcohol	1034	1032	0.1	–
2-Hydroxy benzaldehyde (salicylaldehyde)	1041	1041	0.9	1.5
2-Phenylethanol	1112 ± 1	1110	0.4	1.1
Ethyl benzoate	1172	1170	0.1	–
p-Methyl acetophenone	1178	1182	–	0.2
Eugenol	1358 ± 1	1356	1.5	2.9
(*Z*)-Caryophyllene	1404 ± 1	1404	0.9	0.4
(*E*)-Caryophyllene	1417 ± 1	1418	0.8	trace
β-Humulene (?)	1436	1440	0.3	–
(*Z*)-β-Farnesene	1445	1443	0.1	–
(*E*)-β-Farnesene	1457	1458	0.1	–
(*E*)-Ethyl cinnamate	1463	1462	0.02	–
Sesquiterpenoid $C_{15}H_{24}O$	1474 ± 1	–	0.02	0.9
γ-Curcumene	1480 ± 1	1480	2.1	trace
Ar-Curcumene	1483 ± 1	1483	0.2	trace
β-Selinene	1487	1485	0.1	–
δ-Guaiene (α-bulnesene)	1508	1505	0.4	–
β-Bisabolene	1511	1509	0.1	–
Caryophyllene oxide	1582 ± 1	1581	0.3	0.6
Guaiol	1596 ± 1	1595	13.2	8.7
γ-Eudesmol, 10-*epi*–	1617 ± 1	1619	1.8	0.7
β-Eudesmol (β-selinenol)	1637 ± 2	1649	2.3	1.0
α-Eudesmol	1640	1652	5.0	1.3
Bulnesol	1664 ± 2	1666	9.7	3.8
4-Methoxymethyl cinnamate, (?)	1668	–	–	0.3
Hexadecanal (?)	1811	–	–	0.3
3,4-Dimethoxymethyl cinnamate	1873	–	–	0.5
n-Heneicosane	2100	2100	0.3	0.3
2-Phenylethyl cinnamate (?)	2158	–	–	0.2
n-Docosane	2200	2200	0.1	0.1
n-Tricosane	2300	2300	4.1	9.1
2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-	2365 ± 1	–	0.5	3.0
Cinnamyl cinnamate	2391	–	–	2.4
n-Tetracosane	2400	2400	1.2	1.6
NN	2460	–	–	1.9
n-Pentacosane	2500	2500	9.0	15.9
4-H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl–	2543	–	–	0.5
n-Hexacosane	2600	2600	1.2	1.4
Docosanol acetate	2611	2613	–	0.5
Tetracosanal (?)	2614	–	–	0.1
n-Heptacosane	2700	2700	18.9	18.6
n-Octacosane	2800	2800	0.1	0.4
n-Nonacosane	2900	2900	9.0	3.8
n-Triacontane	3000	3000	1.8	0.05
n-Hentriacontane	3100	3100	2.4	0.9
2-Phenyl ethyl eicosanoate	–	3110*	–	0.5
2-Phenyl ethyl docosanoate	–	3303*	–	0.6
2-Phenyl ethyl tetracosanoate	–	3514*	–	0.6
2-Phenyl ethyl hexacosanoate	–	3724*	–	0.6

* Dr. E. N. Dubis (private communication, 2001).

Table II. Composition of "acidic" compounds of ether extracts from poplar buds.

Compound (TMS-ester)	RI^{exp}	RI^{Lit}	Content, rel. %	
			P. balsamifera	*P. nigra*
3-Methyl butanoic acid	932	932	–	0.1
Ethylene glycol	992 ± 1	–	0.3	0.02
1,2-Propanediol	1010	–	0.2	–
Tiglic acid	1014	1015	–	0.04
Phenol	1052 ± 1	1052	0.9	0.2
Lactic acid	1068 ± 1	1066	1.9	0.3
Benzyl alcohol	1153	1150	0.4	–
2-Phenylethanol	1223	1234	0.3	–
Benzoic acid	1244 ± 2	1249	12.8	0.8
1,2-Cyclohexadiol (?)	1262 ± 1	–	5.8	0.1
1,*n*-Cyclohexadiol (?)	1275 ± 1	–	2.8	0.1
Phosphoric acid	1290	1289	–	0.5
Glycerol	1296 ± 1	1290	12.7	0.9
Pyrocatechol	1321 ± 1	1320	1.3	0.5
Succinic acid	1323 ± 1	1321	0.7	0.1
Dihydrocinnamic (benzenepropanoic) acid	1414 ± 1	1410	1.0	1.7
NN	1441 ± 1	–	2.2	0.8
Eugenol	1472	1454	trace	0.2
Malic (2-hydroxybutanedioic) acid	1509 ± 1	1508	1.0	0.2
Cinnamic acid	1540 ± 1	1540	4.9	0.7
Protocatechuic aldehyde (?)	1618 ± 1	–	2.5	1.0
4-Hydroxybenzoic acid	1629 ± 3	1635	1.1	0.1
4-Hydroxyphenylacetic acid	1644 ± 3	1649	0.6	0.2
4-Methoxy methyl cinnamate	1665	–	–	0.1
Guaiol	1681	–	–	1.2
4-Hydroxyhydrocinnamic acid	1763	1760	–	0.1
4-Methoxycinnamic acid	1825 ± 1	–	1.6	7.1
NN	1852 ± 1	–	2.4	0.6
3,4-Dimethoxy methyl cinnamate	1873 ± 1	–	0.9	0.6
p-Coumaric acid	1943 ± 1	1948	11.0	2.6
3,4-Dimethoxycinnamic acid	2035 ± 3	–	2.5	15.3
Hexadecanoic acid	2053 ± 3	2058	0.2	2.2
Ferulic acid	2096 ± 3	2105	1.7	11.9
Caffeic acid	2152 ± 1	2151	1.0	1.8
(*Z*,*Z*)-9,12-Octadecadienoic acid	2212 ± 2	2230	0.4	1.5
α-Linolenic acid	2218 ± 3	2232	0.4	1.8
NN	2230 ± 1	–	0.3	0.8
Octadecanoic acid	2248 ± 3	2255	1.4	0.4
NN	2322	–	1.4	–
3-Methyl-3-butenyl caffeate	2367	2362	–	2.5
NN	2408	–	–	1.5
3-Methyl-2-butenyl caffeate	2422	2420	–	4.6
Pinostrobin chalcone	2500	2500	–	0.3
3-Methyl-2-butenyl-3-acetyloxycaffeate	2505	2503	–	0.3
Pinocembrin	2544	2542	–	6.7
NN	2602	–	1.6	–
NN	2678	–	1.0	–
Chrysin (2,5-dihydroxyflavone, mono-TMS)	2644	2649	–	1.0
Benzyl caffeate	2740	2735	–	6.7
2-Phenylethyl caffeate	2801 ± 1	2800	0.3	6.9
β-Sitosterol (?)	> 3100	–	1.0	2.6

The third group (RI range 1670–2390) is formed by esters of cinnamic acid. The total content of cinnamates in extracts of *P. nigra* was 3.7% TIC, whereas in *P. balsamifera* buds they not detected even in trace amounts.

About 50% of total ion current in recording the chromatograms of hexane extracts consisted of C_{21}–C_{31} *n*-alkanes. Higher alkanes are known to be one of the main components of cuticular waxes of plant leaves and stems. Moreover, in the homologue series the hydrocarbon fraction with an odd number of carbon atoms predominates considerably. In our case the value of CPI (Carbon Preference Index) (Isidorov, 1990) exceeded 10 for both poplar species.

Recently 2-phenyl ethyl esters of higher carboxylic acids have also detected in cuticular waxes (Gülz and Marner, 1986). In hexane extracts of buds of both poplar species series of homologous esters of this alcohol consisting of eight components were recorded by the SIM (Selective Ion Monitoring) method with the aid of ions with *m/z* 104, 105, and 43. The relative contribution of each of them to TIC was on the level of 0.3–0.5%. Individual identification of these components was not carried out because their RI were not measured.

Table II contains data on the composition of components identified in ether extracts of buds. Its comparison with the composition given in Table I shows that the lists of these compounds almost not overlap. Some of the relative polar "neutral" compounds (2-phenylethanol, eugenol, guaiol and 3,4-dimethoxy methyl cinnamate) were not extracted with hexane completely but partially passed into the ether fraction.

The components detected in ether extracts can also be divided into several groups. One of them consists of polyols: diols and glycerol. Their contents differ greatly; in extracts of *P. balsamifera* buds glycerol is one of the main components (12.7% TIC), whereas its content in *P. nigra* did not contain 1%.

The main fraction of the ether extract in buds of both poplar species consists of acidic compounds of aliphatic and aromatic series. The former are represented by saturated and unsaturated mono-, dicarboxylic and hydroxycarboxylic acids. Their total content was approximately equal and was on the level of 6% TIC. Aromatic acids are represented by two groups of compounds. One of them includes benzoic, 4-hydroxybenzoic, and 4-hydroxyphenyl acetic acids. The other one is formed by cinnamic acid and its derivatives.

Benzoic acid was one of the major components in extracts from *P. balsamifera* buds (12.8% TIC) but one of the minor components of *P. nigra* buds. The total content of cinnamic acids for both poplar species was approximately equal (21–27% TIC), although the differences in individual composition were considerable. For instance, the extracts from *P. balsamifera* buds contained an almost five times greater amounts of *p*-coumaric acid than that *P. nigra*, whereas the content of caffeic acid was seven times smaller.

The most pronounced difference is evidently observed in the contents of caffeates and flavonoids. In the case of *P. nigra* the total content of pentyl-, benzyl-, and 2-phenylethyl caffeates was 21% TIC, whereas they were almost completely absent in the extract of *P. balsamifera.* Three flavonoids (pinostrobin chalcone, pinocembrin and chrysin) formed 8% TIC of the *P. nigra* extract but were also completely absent in the second poplar species.

Conclusions

Table III gives the group composition of hexane and ether extracts from buds of the two poplar species discussed here. The analysis of these data shows that chemotaxonomic differences are more-

Table III. Group composition (% TIC) of organic compounds in bud extracts of *P. balsamifera* and *P. nigra.*

Group of compounds	*P. balsamifera*	*P. nigra*
Hexane extracts		
Aromatic C_6-C_{10} compounds	3.0	5.7
Sesquiterpene hydrocarbons	5.1	trace
Sesquiterpenoids	32.1	17.9
Cinnamic acid derivatives	–	3.7
C_{21}-C_{31} *n*-Alkanes	47.9	52.1
Ether extracts		
Diols and glycerol	19.1	1.0
Aliphatic acids and hydroxy acids	6.0	6.7
Phenol carboxylic acids	15.5	2.8
Cinnamic acid and their derivatives	21.3	27.1
Caffeates	0.3	21.0
Flavonoids	–	8.0

pronounced when the composition of "acidic" components are compared. However, they are also observed for "neutral" components. The extraction by a non-polar solvent made it possible to broaden greatly the list of components detected previously in buds of both poplar species. Moreover, sample preparation for extraction with hexane is much less laborious and reagents used for derivatization are not needed.

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