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VOLATILE CONSTITUENTS OF BALSAM POPLAR: THE PHENOL GLYCOSIDE CONNECTION

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Key Word Index—*Populus balsamifera*; Salicaceae; balsam poplar; phenol glycosides; 6-hydroxycyclohex-2-enone; cyclohexan-1,2-dione; salicortin; trichocarpin; trichocarpigenin.

Abstract—The volatile metabolites of balsam poplar winter-dormant buds are a complex array of mono- and sesquiterpenoids with 1,8-cineol, *trans*-nerolidol and (+)-(1*R*,1′*R*)-α-bisabolol being the major components. On the other hand the volatiles of internodes (stems between buds) consist mainly of salicaldehyde and (+)-6-hydroxycyclohexanone with minor amounts of cyclohexan-1,2-dione and an unidentified compound. This is the first report of these cyclohexanones as natural products; salicortin, a phenol glycoside, appears to be their biosynthetic precursor. A second phenol glycoside, trichocarpin, is present in poplar internodes; and it is the progenitor of trichocarpigenin (benzyl gentisate), an easily formed artifact.

INTRODUCTION

There has been a longstanding interest in the chemistry of poplars. The major impetus for this activity has been that poplars have considerable economic potential in that they have high growth rates [1], are utilized for the production of pulp [2], and contain leaf and bud oils with useful biological [3, 4] and aesthetic [5] properties. The dietary importance of poplars for wild herbivores in high latitude forests has also attracted the attention of chemical ecologists [6].

Given the chemical interest in poplars and the wide geographical distribution of balsam poplar (*Populus balsamifera* L.), it is not surprising to find that considerable information is available on the chemistry of this species. In a lengthy series of papers on the Salicaceae, Pearl and Darling described a variety of water-soluble phenols, carboxylic acids and glycosides from various parts of this plant [7]. Flavonoids and chalcones have been reported [8] as have fatty acids [9] and aliphatic alcohols [10]. Buds of balsam poplar are known to be a rich source of mono- and sesquiterpenes [11], although the chemical composition of bud oil has not been fully described. There are even recent reports of prostaglandins in this species [12, 13].

Our interest in balsam poplar has focused on the relationship between its complex chemistry and the selective utilization of this tree by a variety of herbivores, especially the snowshoe hare [14, 15]. We have thus initiated our own study of balsam poplar and report here our findings on volatile metabolites from Alaskan populations of this species.

RESULTS

Volatile components of the bud oil of winter-dormant P. balsamifera

Analysis of the steam distillate from *P. balsamifera* buds by GC/MS revealed a mixture of over 20 components (Table 1). GC/MS analysis of the dichloromethane extract of buds also revealed the components of Table 1 (with additional components of longer retention times), indicating the absence of artifacts in the steam distillate.

Structures were assigned to all of the components (1–8, Table 1) eluting in the first portion of the chromatogram (1–12 min) from their mass spectra, and assignments were confirmed by similar analyses of authentic compounds. With the exception of benzyl alcohol all compounds eluting in this portion of the chromatogram are monoterpene alkenes, alcohols or ethers.

The middle portion of the chromatogram (15–29 min) consisted of a series of compounds (9–16, Table 1) which, according to their fragmentation patterns, are sesquiterpenes. However, no definitive assignments of structure can be made at this time.

The final portion of the chromatogram (> 29 min) consisted of sesquiterpene alcohols (17–22, Table 1). The two major components of this series of compounds were isolated and fully characterized as *trans*-nerolidol and (+)-(1*R*,1′*R*)-α-bisabolol. The relative stereochemistry of the bisabolol was assigned by ^{1}H NMR spectroscopy [16] and its absolute configuration from polarimetry data [17]. This stereochemical assignment is consistent with that proposed by Flaskamp *et al.* [18] for bisabolol isolated from European *P. balsamifera*.

Volatile components from internodes of winter-dormant P. balsamifera

GC analyses of ether and dichloromethane extracts of fresh internodes (portions of stem between buds) of *P. balsamifera* displayed four peaks with relatively short retention times as well as a major component with a much longer retention time. The two most abundant components with short retention times proved to be salicylaldehyde (1) and (+)-6-hydroxycyclohexenone (2). In each case the compound was isolated and its spectral as well as chromatographic properties were compared with those of authentic material—a commercial sample of 1

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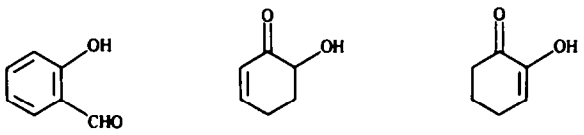
Table 1. Components of steam distillate from buds of *P. balsamifera*

Peak no.	Name	M±*
1	α-Pinene	136
2	β-Pinene	136
3	Myrcene	136
4	Cineol†	154
5	Benzyl alcohol	108
6	γ-Terpinene	136
7	Terpinen-4-ol	154
8	α-Terpineol	(154)
9	α-Bergamotene‡	204
10	β-Farnesene†‡	204
11	Unknown	202
12	Unknown	(?)
13	Unknown	204
14	δ-Cadinene†‡	204
15	Unknown	(?)
16	Unknown	204
17	*trans*-Nerolidol	222
18	Unknown	(?)
19	Unknown	222
20	Unknown	(?)
21	Unknown	222
22	α-Bisabolol†	222

*Fragment ions and relative intensities of identified components agreed with lit. data.

†Previously reported as a constituent of *P. balsamifera* buds by Sôrm *et al.* [11].

‡Assignment (tentative) based only upon analysis of mass spectrum.



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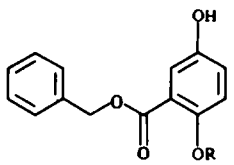

4 R = H
5 R = Glc

and a synthetic [19] sample of **2**. One minor component has not yet been identified, but the other has been characterized as cyclohexan-1,2-dione (**3**) by comparison with a commercial sample. The long retention time component has been fully characterized as benzyl gentisate (trichocarpigenin, **4**) by comparison with synthetic [20] material. However, we believe that **4**, a previously reported constituent of *P. balsamifera* bark [21], is an artifact produced during the extraction process (see below).

Since we were unable to find previous reports of **2** and **3** as natural products and since they have rather unusual structures from a biosynthetic standpoint, we conducted several experiments to verify their authenticities as natural products. Both compounds, as well as **1**, can be detected by TLC as well as GC in the ether extract of fresh plant material. They can also be extracted from dried plant material, although their concentrations are reduced presumably due to evaporative loss during lyophilization. Furthermore they can be obtained by mild distillation (25°/0.07 mm) of fresh plant material.

In our efforts to obtain large enough samples of **1**, **2** and **3** for spectroscopic characterization we carried out steam distillation of fresh poplar internodes. The gas chromatographic trace of the steam distillate displayed, in addition to peaks for each of the four most volatile components of the ether extract, several new peaks. The most abundant artifact produced during steam distillation proved to be phenol, and quantitative analyses of the ether extract and steam distillate suggested that it had been produced at the expense of **2**. We subsequently discovered that samples of **2** slowly convert to phenol upon storage and that the conversion is greatly accelerated in acidic media.

Phenol glycosides from the internodes of P. balsamifera

The presence of **4** in balsam poplar internodes prompted us to search for trichocarpin (**5**), a glucoside of **4** which has previously been reported as a metabolite of *P. balsamifera* [21, 22]. Chromatographic examination of an aqueous extract of poplar internodes revealed the presence of two major components. One of them proved to be **5** as shown by physical and spectral data and its acid-catalysed hydrolysis to **4** [23]. The other was identified as salicortin (**6**), another known metabolite of *P. balsamifera* [22], by comparison of physical and spectral properties with those cited in the literature [23].

However, a troubling aspect of these findings was that quantitative analysis of the ether extract indicated that fresh poplar internodes contain about 3% of **4** and examination of the aqueous extract indicated the plant contains similar amounts of **5** (Table 2). It seemed to us unlikely that the plant would contain such high, nearly equal concentrations of both metabolites.

We therefore tested the hypothesis that **4** is an artifact, produced from **5** during extraction. This hypothesis was verified by the following findings: (1) while the ether extract of fresh *P. balsamifera* internodes contained **4** (TLC), an anhydrous ether extract of freeze-dried internodes did not; (2) repetition of the later extraction modified by the addition of water (a required reactant for hydrolysis) produced an extract containing **4**; (3) the ether extract of fresh material which had previously been heated at 75° for 1.5 hr did not contain **4**. We interpret these results to mean that not only is **4** an artifact but that it is produced enzymatically during the course of extraction. Most likely **5** and an enzyme which catalyses its hydrolysis to **4** are segregated from one another in the plant. Cell rupture or organelle disruption during the extraction allows them to come in contact; and, in the presence of water, the glucoside is hydrolysed. A second possibility is that the enzymatic hydrolysis of **5** is accelerated when ether serves as the extracting solvent [24].

We hoped to gain further support for this conclusion by demonstrating β-glucosidase activity in *P. balsamifera* internodes. Thus a sample of **5** was incubated with an

enzyme preparation made from winter-dormant internodes according to the procedure of Seigler *et al.* [25]. No **4** was detected by TLC, indicating the absence of an appropriate β-glucosidase in this preparation. While we have been unable to demonstrate the conversion of **5** to **4** using this enzyme preparation from balsam poplar, we believe the results cited above together with the thermal and hydrolytic stability of **5** require that **4** is enzymatically produced from **5** during extraction of fresh plant material.

In contrast to the stability of **5** under these conditions when **6** was incubated with the enzyme preparation, production of salicin (**7**) was evident by TLC, and GC/MS analysis showed the production of **2** and **3**. No **2** or **3** was produced from a similar incubation of **6** with a boiled enzyme preparation. These findings together with the previous reports of different products from the abiotic hydrolysis of **6** [7] strongly suggest that the observed conversion of **6** to **2** and **3** is the result of an enzyme-catalysed process.

Quantitative analyses

The concentrations of the major volatile secondary metabolites of balsam poplar have been quantified in saplings growing under a variety of natural and environmentally manipulated conditions in interior Alaska, and the findings are summarized in Table 2.

DISCUSSION

One salient feature of our results is the striking difference between secondary metabolism in the buds and internodes of balsam poplar. Volatile bud metabolites come almost exclusively from the isoprenoid pathway, while internode chemistry seems to be dominated by the shikimate route. Although the localization of production and storage of individual secondary metabolites within plant parts is not uncommon [26], balsam poplar expresses an unusual degree of such localization.

The enzymatic conversion of **6** to **2** and **3** presumably reveals the final steps in the biosyntheses of these novel compounds. One possible mechanism for this conversion (Fig. 1) entails ester hydrolysis to give the putative carboxylic acid **8** which decarboxylates to **9**, the immediate precursor of **2** and **3**. Two features of this mechanism are noteworthy. The intermediacy of **8** in the enzymatic process is consistent with Pearl and Darling's [7] proposal that it (in its conjugate base form) is involved in the abiotic alkaline conversion of **6** to pyrocatechol. Secondly, if Fig. 1 accurately portrays the pathway, then the isomerization of **9** to **2** must also be an enzymatic process in that **9** is achiral while **2** isolated from balsam poplar is optically active. Even though these findings shed light on the final stages of the biosynthetic pathways to **2** and **3**, they lead to the interesting question of the origin of the aliphatic portion of salicortin.

The levels of individual secondary metabolites in balsam poplar saplings exhibit only moderate variations among individual trees, with neither populational nor environmental variables seeming to have major effects on the production of these compounds (see Table 2).

Finally we note that the findings reported here on localization of metabolites in balsam poplar, enzymatic and abiotic reactions of certain poplar metabolites, as well as moderate differences in concentrations of individual compounds between trees which are genetically different and/or exist in different environmental situations all have substantial ecological implications. These will be addressed in a forthcoming publication.

EXPERIMENTAL

Gas chromatography (GC). GC/MS (EI, 70 eV) analyses employed a WCOT polydimethylsiloxane capillary column (25 m × 0.21 mm i.d.) with a temperature programme of T_1 = 50°/2 min, 50–280° at 4°/min. All analytical work (FID) was done with an OV-330 column (2 m × 2 mm i.d.) using one of two temperature programmes: T_1 = 80°/16 min, 80–200° at 8°/min, T_2 = 200°/16 min (bud essential oil); T_1 = 85°/16 min, 85–230° at 8°/min, T_2 = 230°/32 min (internode extracts). Preparative GC was done with an OV-17 column (6 m × 0.8 cm i.d.) at 175°.

Flash chromatography. Flash chromatography was carried out following the procedure of Still *et al.* [27].

Thin layer chromatography (TLC). Precoated EM silica gel and Whatman $MKC_{18}F$ (reverse phase) plates with fluorescent indicator were used. Components were detected with a 254 nm

Table 2. Levels of secondary metabolites of balsam poplar

Compound	Analytical method	Plant part	Percentage of plant mass*
Salicaldehyde	†	Internode	0.05–0.60
6-Hydroxycyclohexenone	†	Internode	0.2–0.6
Benzyl gentisate	‡	Internode	1.5–3.5§
Trichocarpin	—	Internode	3.5‖
Salicortin	—	Internode	4.0‖
Cineol	†	Bud	0.3–0.7
Benzyl alcohol	†	Bud	0.3–0.6
α-Bisabolol	†	Bud	1.3–2.6

Concentrations are given as ranges and are expressed as a percentage of fresh plant mass.

*Ranges given are the extreme values found in a survey of several hundred plants growing in natural and manipulated (e.g. fertilized) environments at various sites in interior Alaska.

†GC.

‡HPLC.

‖Isolated yield in a single experiment.

§Artifact.

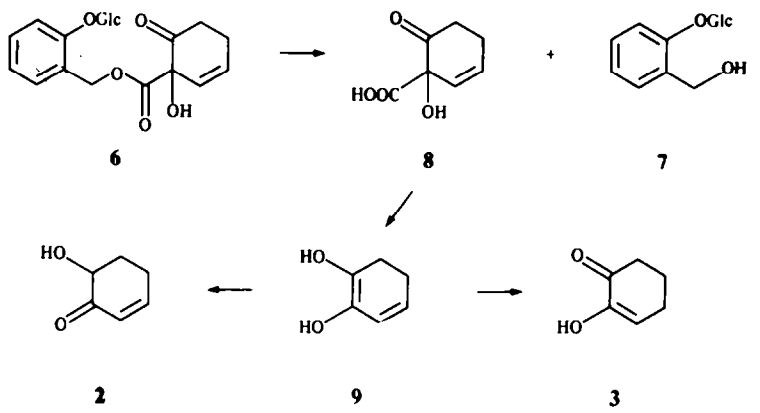

Fig. 1. Proposed biogenetic relationships between salicortin (6), 6-hydroxycyclohexenone (2) and cyclohexan-1,2-dione (3).

lamp, $FeCl_3$ or H_2SO_4/charring. TLC monitoring of flash chromatography columns employed the same solvent system used to develop the column.

Steam distillate from buds. Winter dormant foliar buds were removed from sapling balsam poplar trees collected in the vicinity of Fairbanks, AK. The buds (540 g) were steam distilled until a total of 20 l of distillate was collected. Extraction with CH_2Cl_2 followed by drying ($MgSO_4$) and flash evaporation of solvent gave bud essential oil (17.0 g).

This oil was fractionally distilled using a 15 cm Vigreaux column, and fractions were analysed by GC/MS: fraction 1 (25–35°/0.07 mm, 1.63 g) was mainly 1,8-cineol contaminated with monoterpenes. Fraction 2 (46–65°/0.07 mm, 0.65 g) was principally terpinen-4-ol and α-terpineol. Fraction 3 (66–90°/0.07 mm, 2.81 g) was a complex mixture of sesquiterpenes, including minor amounts of those found mainly in fraction 4. Fraction 4 (90–95°/0.07 mm, 2.90 g) contained principally a mixture of four sesquiterpene alcohols. Residue in the distillation flask consisted of three of the four components found in fraction 4.

Nerolidol. Material (4.6 g) from fraction 4 of the vacuum distillation of bud steam distillate was separated by medium pressure chromatography (Merk RP-8 LoBar column; 80% aq. Me_2CO) followed by flash chromatography (silica gel; petrol–Et_2O, 85:15). The major component was identified as *trans*-nerolidol (0.15 g): ^{1}H NMR (60 MHz; $CDCl_3$): δ6.0–4.8 (5H, *m*, =CH–), 1.56 (3H, *s*, Me), 1.63 (6H, *s*, Me × 2), 1.15 (3H, *s*, Me). IR ν_{max}^{neat} cm^{-1}: 3420 (*br*), 2970, 2925, 1647, 1108, 938. MS, *m/z* (rel. int.): 204 (1), 189 (1), 161 (8), 107 (27), 93 (52), 69 (100).

(+)-(1R,1R')-α-*Bisabolol.* A portion (2.5 g) of the residue from fractional distillation of bud steam distillate was separated by flash chromatography (silica gel; Me_2CO–CH_2Cl_2, 3.5:96.5). The major component (1.1 g) was rechromatographed under the same conditions and identified as (+)-(1*R*,1'*R*)-α-bisabolol: ^{1}H NMR (250 MHz, $CDCl_3$): δ5.39 (1H, *br s*, =CH–), 5.12 (1H, *t*, =CH–), 1.68, 1.64, 1.62, 1.10 (all 3H, *s*, Me). IR ν_{max}^{neat} cm^{-1}: 3440, 2990, 2885, 1450, 1380. MS, *m/z*, (rel. int.): 222 (1), 204 (3), 189 (1), 161 (3), 121 (19), 109 (39), 95 (19), 93 (27), 79 (16), 69 (100). $[\alpha]_D^{25}$ = +47° (MeOH, *c* 1.25).

Dichloromethane extract of buds. Buds (9.0 g) of balsam poplar were allowed to stand in CH_2Cl_2 (100 ml) for 24 hr at 27°. The solvent was decanted, dried ($MgSO_4$) and flash evaporated to 10 ml. The concentrated extract was analysed by GC/MS.

Ether extract of internodes. Internodes were prepared by cutting winter-dormant twigs of balsam poplar about 1 cm from the bud scar. The internodes (326 g) were extracted with Et_2O (3.26 l.) for 8 days at 27°. The extract was filtered, dried ($MgSO_4$) and evaporated to dryness. A portion (3.89 g) of the extract (30.4 g) was separated by flash chromatography (silica gel; Me_2CO–CH_2Cl_2, 3.5:96.5), and 14 50 ml fractions were collected.

Salicylaldehyde (1). This (0.243 g) was recovered from fractions 1–3: ^{1}H NMR (60 MHz, $CDCl_3$): δ11.0 (1H, *s*, OH), 9.9 (1H, *s*, CHO), 7.7–6.9 (4H, *m*, Ar). IR ν_{max}^{neat} cm^{-1}: 3650–3000 2850, 1670, 1585, 1150. MS, *m/z* (rel. int.): 122 (9), 105 (11), 93 (100).

Benzyl gentisate (4). Recrystallization (CCl_4) of the solid obtained from fractions 4–7 gave 4 (0.254 g): mp 104.5°, lit. 104–105° [20]; ^{1}H NMR (250 MHz, $CDCl_3$): δ10.5 (1H, *s*, OH), 7.50–7.36 (5H, *m*, Ar), 7.35 (1H, *d*, *J* = 3.5 Hz, C-6), 7.00 (1H, *dd*, *J* = 9, 3.5 Hz, C-4), 6.90 (1H, *d*, *J* = 9 Hz, C-3), 5.40 (2H, *s*, $-CH_2O-$), 5.20 (1H, *s*, OH); ^{13}C NMR (62.5 MHz, $CDCl_3$): δ169.5 (*s*), 155.8 (*s*), 147.8 (*s*), 135.2 (*s*), 128.7 (*d*, × 2), 128.6 (*d*), 128.2 (*d*, × 2), 124.2 (*d*), 118.5 (*d*), 112.2 (*s*), 67.1 (*t*).

A sample of 4 (99 mg) was saponified in ethanolic KOH. Analysis of the neutral fraction by GC showed a single peak which coincided with that for authentic benzyl alcohol. The acidic material from saponification was recrystallized from CCl_4 to give gentisic acid (8 mg): IR ν_{max}^{Nujol} cm^{-1}: 3300, 1660, 1590, 1200, 934, 855, 790, 785, 750, 720, 680.

(+)-6-*Hydroxycyclohexen-2-one* (2). Fractions 8–11 contained an oil mixed with 4. Kugelrohr distillation (90–110°/12 mm) gave 2 (oil): ^{1}H NMR (60 MHz, $CDCl_3$): δ7.3–6.8 (1H, *m*, C-3), 6.1 (1H, *br d*, *J* = 10 Hz, C-2), 5.20 (*br s*, OH), 4.5–4.1 (1H, *dd*, *J* = 13, 7 Hz, C-6), 2.7–1.5 (4H, *m*, C-4, 5). IR ν_{max}^{neat} cm^{-1}: 3470, 1685, 1620, 1105. MS, *m/z* (rel. int.): 112 (28), 84 (29), 68 (10), 67 (100). $[\alpha]_D^{27}$ = +33° (95% EtOH, *c* 3.9).

A sample of 2 (100 mg) was dissolved in Me_2CO-d_6 (0.4 ml) and a drop of conc HCl added. Monitoring of the ^{1}H NMR (60 MHz) signals at *ca* δ6.0 and δ6.7–7.1 indicated the conversion of 2 to phenol had $t_{1/2}$ of *ca* 30 min at 27°.

1,2-*Cyclohexanedione* (3). Kugelrohr distillation (90–120°/2 mm) of a CH_2Cl_2 extract of poplar internodes gave a clear oil. A portion (4.50 g) of this oil was separated by flash chromatography (silica gel; Me_2CO–CH_2Cl_2, 3.5:96.5) and the fractions containing a mixture of 2 and 3 were rechromatographed in this system to give 3 (0.40 g) as an oil which solidified upon standing: ^{1}H NMR (60 MHz, CCl_4): δ5.97 (1H, *t*, *J* = 6 Hz, C-3), 2.5–1.0 (7H, *m*). IR ν_{max}^{neat} cm^{-1}: 3420, 2900, 1665, 1645, 1220, 1885. MS, *m/z* (rel. int.): 112 (16), 83 (16), 70 (100), 69 (41), 55 (31).

Synthesis of benzyl gentisate (4). A suspension of Na

gentisate·2.5H_2O (22 g) in a soln of benzyl bromide (84 g) and Me_2CO (900 ml) was refluxed for 19 hr. After most of the Me_2CO was distilled from the reaction mixture, Et_2O was added and the organic soln extracted with 5% aq. NaOH. The aq. soln was acidified and extracted with Et_2O. After washing with satd $NaHCO_3$ soln and drying ($MgSO_4$), the ether soln was evaporated to give a dark solid (15 g). Recrystallization (Me_2CO–CCl_4; −20°) gave **4** (6.7 g, 27%). Flash chromatography (silica gel; Et_2O–petrol, 3:2) of the residue from mother liquors followed by recrystallization of appropriate fractions gave additional **4** (2.3 g, 11%).

Synthesis of 6-hydroxycyclohexenone (**2**). Cyclohexenone (4.32 g) was converted to **2** (1.87 g, 39%) by the procedure of Rubottom and Gruber [19].

Aqueous extract of balsam poplar internodes. Dried and ground internodes (5.0 g) of *P. balsamifera* were extracted in boiling H_2O (50 ml) for 5 min and filtered. The extract was freeze-dried to afford a tan solid (1.3 g). Two separate portions (0.5 g each) of this solid were separated by flash chromatography (Baker RP-C18; 20% aq. Me_2CO followed by Me_2CO). Twenty 10 ml fractions were collected from each column, and appropriate fractions (TLC monitoring) from both columns were combined.

Salicortin (**6**). After an initial fraction (0.27 g) containing uncharacterized carbohydrates, fractions 5–7 were combined and evaporated to give **6** (0.31 g) as an amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ7.30 (2H, *m*, Ar), 7.21 (1H, *d*, J = 7 Hz, Ar), 7.02 (1H, *t*, J = 7 Hz, Ar), 6.11 (1H, *dt*, J = 9.4, 3.7 Hz, C-5), 5.77 (1H, *br d*, J = 9.4 Hz, C-6), 5.34 (1H, *d*, J = 13 Hz, benzylic), 5.22 (1H, *d*, J = 13 Hz, benzylic), 4.98 (1H, *d*, J = 8 Hz, C-1″), 3.88 (1H, *m*), 3.80 (1H, *t*, J = 8 Hz), 3.70 (1H, *m*), 3.50 (3H, *m*), 2.97 (4H, *s*), 2.94 (1H, *s*), 2.85 (1H, *m*, C-4), 2.65 (1H, *m*, C-4), 2.52 (2H, *m*, C-3). ^{13}C NMR (125 Mz, DMSO-d_6): δ206.1 (*s*), 170.2 (*s*), 155.1 (*s*), 131.7 (*d*), 129.5 (*d*), 128.9 (*d*), 128.4 (*d*), 124.6 (*s*), 121.9 (*d*), 115.1 (*d*), 101.0 (*d*), 77.5 (*s*), 77.2 (*d*), 76.6 (*d*), 73.4 (*d*), 69.9 (*d*), 62.3 (*t*), 60.9 (*t*), 35.7 (*t*), 26.0 (*t*).

Trichocarpin (**5**). After **6** had eluted, the solvent was changed to Me_2CO. Fractions 9–15 were combined and evaporated to afford **5** (0.27 g): mp 133–134° (H_2O), lit. 135–136° [28]; ^{1}H NMR (500 MHz, Me_2CO-d_6): δ8.56 (1H, *s*, OH), 7.51 (2H, *d*, J = 7 Hz, C-2′,6′), 7.40 (2H, *t*, J = 7 Hz, C-3′,5′), 7.35 (1H, *m*, C-4′), 7.32 (1H, *d*, J = 7 Hz, C-3), 7.25 (1H, *d*, J = 2 Hz, C-6), 7.00 (1H, *dd*, J = 7, 2 Hz, C-4), 5.33 (1H, *d*, J = 13 Hz, benzylic), 5.30 (1H, *d*, J = 13 Hz, benzylic), 4.70 (1H, *d*, J = 8 Hz, C-1″), 3.90 (1H, *m*), 3.80 (1H, *t*, J = 7 Hz), 3.70 (1H, *m*), 3.45 (3H, *m*), 2.99 (3H, *s*), 2.96 (1H, *s*). ^{13}C NMR (125 MHz, Me_2CO-d_6): δ168.0, 154.7, 138.2, 130.5, 130.3, 130.2, 124.1, 122.9, 122.8, 122.7, 118.5, 118.4, 106.8, 79.3, 78.5, 76.1, 72.4, 68.7, 63.9.

Trichocarpin (200 mg) was dissolved in 10 ml of 1.0 M HCl and heated to 70° for 10 min. The reaction mixture was extracted with Et_2O and the extract dried ($MgSO_4$). Analysis of the concentrated extract by TLC (silica gel; petrol–Et_2O, 3:2) revealed the presence of **4**.

Benzyl gentisate (**4**) *as an artifact in balsam poplar.* Finely ground fresh internodes of balsam poplar (0.5 g) were allowed to stand in Et_2O (15 ml) for 24 hr. The dried ($MgSO_4$) extract was concentrated under vacuum. TLC analysis (silica gel; petrol–Et_2O, 3:2) indicated the presence of **4** in the extract.

A similar extraction of ground freeze-dried internodes (0.5 g) with anhydrous Et_2O provided an extract containing no **4** (TLC). Repetition of this experiment with the addition of H_2O (0.5 ml) to the extraction provided an extract containing **4** (TLC).

Fresh poplar twigs (9.2 g) were heated to 75° for 1.5 hr and then extracted with Et_2O for 3 days. TLC analysis revealed no **4** in the concentrated extract.

Enzyme preparation. An enzyme preparation from *P. balsamifera* was made basically according to the procedure of Seigler *et al.* [25]. Freshly cut winter-dormant internodes were freeze-dried and ground to a fine powder. A portion (16 g) of the powder was allowed to stand in Me_2CO (100 ml) at 0° for 1 hr. After filtration and washing with cold Me_2CO the powder was extracted with 200 ml of 0.02 M phosphate buffer (pH = 6.8) containing 0.01% NaN_3 at 0° for 1 hr. The aq. soln was twice dialysed against buffer (2.5 l.) for 12 hr at 0°. The contents of the dialysis bag were centrifuged (5000 rpm, 10 min) and the supernatant enzyme preparation decanted and stored at 5°.

Incubation of trichocarpin (**5**) *with enzyme preparation.* Trichocarpin (40 mg) was incubated with enzyme preparation (15 ml) at 27°. After 12 hr the incubation was extracted with Et_2O and the organic phase dried ($MgSO_4$) and concentrated. TLC analysis of the concentrated extract revealed no **4**.

Incubation of salicortin (**6**) *with enzyme preparation.* Salicortin (50 mg) was incubated with enzyme preparation (15 ml) as described for **5**. Monitoring of the reaction mixture by TLC in two systems (silica gel: $CHCl_3$–MeOH, 4:1; reverse phase: 50% aq. Me_2CO with 0.5% NaCl) showed the production of **7**. Analysis of a concentrated Et_2O extract of the reaction mixture by GC and GC/MS demonstrated the presence of **2** and **3**. A similar experiment with enzyme preparation that had been heated to 100° for 10 min produced no **2** or **3** (GC). To this point we have been unable to obtain an enzyme preparation capable of the conversion of **6** to **2** and **3** from poplar internodes harvested during the growing season.

Steam distillation of balsam poplar internodes. Poplar twigs (269 g) were cut *ca* 2 cm on either side of the bud scars, and the internodes were subjected to steam distillation. The distillate (16 l.) was extracted with CH_2Cl_2. The organic phase was dried ($MgSO_4$) and concentrated under vacuum to a dark brown oil (7.5 g). Analytical GC of the oil showed the presence of **1**, **2**, **3** and a major component (phenol) of longer retention time.

Isolation of phenol from steam distillate of poplar internodes. Samples of **1**, **2**, **3** and the long retention time component (retention times of 6, 7, 5.5, and 11 min, respectively) of the steam distillate were collected by prep. GC from repetitive injections of 0.1 ml of the oil from steam distillation. The long retention time component was identified as phenol: mp 38–40°; ^{1}H NMR (60 MHz, $CDCl_3$): δ7.0 (5H, *m*, Ar), 4.9 (1H, *s*, OH). IR ν_{max}^{KBr} cm^{-1}: 3300, 1600, 1500, 1475, 1230,. MS, *m/z* (rel. int.): 94 (100), 66 (38), 55 (27).

Direct detection of volatiles from poplar internodes. Fresh poplar internodes (60 g) were cut into 1 cm lengths and divided into two subsamples of 30 g each. One subsample was extracted in Et_2O for 1 week. The other portion was evacuated at 35°/0.7 mm for 5 hr. The concentrated Et_2O extract and the dried ($MgSO_4$) distillate were each diluted to 50 ml with $CHCl_2$ and analysed by GC. Compounds **1**, **2** and **3** were all detected in both samples with their concentrations in the Et_2O extract 2–3 × those in the Kugelrohr distillate. Similar GC analysis of a CH_2Cl_2 extract of freeze-dried internodes also revealed the presence of **1**, **2** and **3**.

Quantitative analyses. Cineol, benzyl alcohol and α-bisabolol. Foliar buds of winter-dormant balsam poplar were removed, weighed (0.2–2.0 g), and allowed to stand in CH_2Cl_2 (10 ×, v/w) containing *d*-camphor as an internal standard for 48 hr. Anhydrous $MgSO_4$ was added and the samples were filtered. The extracts were allowed to evaporate in a fume hood until an appropriate vol. (*ca* 0.5 ml) was attained for analysis by GC.

6-Hydroxycyclohexenone (**2**). Carefully weighed internodes (0.7–1.0 g) were allowed to stand in CH_2Cl_2 (10 ×, v/w) containing γ-terpinene as an internal standard for 9 days. The extract was processed by the procedure described for buds and analysed by GC.

Salicaldehyde (1). Fresh internodes (0.7–1.0 g) were extracted with Et_2O and analysed by the procedure described for 2.

Benzyl gentisate (4). Fresh internodes (0.7–1.0 g) were extracted with Et_2O, filtered and the extract diluted to 25.0 ml with Et_2O. Internal standard (synthetic pinosylvin dimethyl ether) [29] was added to a 1.0 ml portion of each extract, and the samples were analysed by HPLC (C_{18}; 60% aq. MeCN).

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