Chemical composition of the bark essential oil of *Cercis canadensis* L. (Fabaceae)

Kelly Marie Steinberg, Prabodh Satyal and William N Setzer

Abstract

The volatile components from the bark of *Cercis canadensis* L. (Fabaceae) were obtained by hydrodistillation and analyzed by gas chromatography–mass spectrometry as well as enantioselective gas chromatography. The bark volatiles were dominated by C6 fatty-acid-derived compounds 1-hexanol (23.3%), hexanoic acid (18.2%), and (2E)-hexenoic acid (3.4%). The concentration of monoterpenoids in *C. canadensis* bark was low (4.1%), but did allow determination of the enantiomeric distribution of α-pinene (racemic), limonene (exclusively d-enantiomer), linalool and α-terpineol (predominantly l-stereoisomers).

Keywords: Essential oil composition, *Cercis canadensis*, enantiomeric distribution, Native American ethnopharmacology

1. Introduction

*Cercis canadensis* L. (Fabaceae), commonly known as “eastern redbud”, ranges throughout the southeastern United States. The tree was used by Native Americans for food as well as medicine. The bark of *C. canadensis* was used to make a tea as a remedy for whooping cough (pertussis), congestion, fever, and vomiting [1]. As part of our continuing interest in Cherokee traditional medicines [2-5], we have investigated the essential oil composition, including enantiomeric distribution of monoterpenoids, of the bark of *C. canadensis*. To our knowledge, this is the first examination of the essential oil of *C. canadensis* and the first report of enantiomeric distribution of monoterpenoids in the Fabaceae.

2. Materials and Methods

2.1 Plant Material

Branches of *C. canadensis* were collected from Huntsville, Alabama (34° 38′ 46″ N, 86° 33′ 27″ W, 191 m elevation) on November 5, 2016. The bark was stripped from the limbs and finely chopped. The chopped bark (87.78 g) was hydrodistilled using a Likens-Nickerson apparatus, with continuous extraction with dichloromethane, for 4 h. *C. canadensis* bark essential oil (1.6709 g, 1.904% yield) was obtained as a colorless liquid, which was stored at –20 °C until further analysis.

2.2 Gas Chromatography – Mass Spectrometry

GC-MS analysis was carried out using a Shimadzu GCMS-QP2010 Ultra. This instrument was operated in the electron impact (EI) mode set at electron energy 70eV with a scan range of 40-400 amu, a scan rate of 3.0 scans per second, and with GC-MS solution software. A ZB-5 fused silica capillary column with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 μm was used as the GC column. Helium was used as the carrier gas and the pressure was set at 80 psi with a flow rate of 1.37 mL/min on the column head. The temperature of the injector was set at 250 °C and the temperature of the ion source was set at 200 °C. The temperature of the GC oven was programmed to be 50 °C initially and was programmed to increase at a rate of 2 °C/min to a final temperature of 260 °C. The sample was prepared with CH2Cl2 in a 5% w/v solution. Then, 0.1 μL of the solution was injected into the instrument with the splitting mode with a split ratio of 30:1. The retention indices were determined by reference to a homologous series of n-alkanes. The components of each essential oil sample were identified based on their retention indices and mass spectral fragmentation patterns compared to reference literature [6] and our in-house library.
2.3 Chiral Gas Chromatography – Mass Spectrometry

The essential oil from *C. canadensis* was also analyzed enantioselectively with a Shimadzu GCMS-QP2010S. The instrument was operated in the EI mode with electron energy of 70 eV, a scan range of 40–400 amu, and a scan rate of 3.0 scans/s. The capillary column used was a Restek B-Dex 325 with film dimensions of 30 m × 0.25 mm ID × 0.25 μm. The temperature of the oven was programmed to start at 50 °C and rise at a rate of 1.5 °C/min to a final temperature of 120 °C. Then, the oven was raised to 200 °C at a faster rate of 2 °C/min and maintained for 5 min. The carrier gas, helium, was set at a constant flow rate of 1.8 mL/min. A solution (0.1 μL) of 3% w/v of the essential oil in CH2Cl2 was injected into the instrument in split mode with the split ratio of 1:45.

3. Results and Discussion

The composition of *C. canadensis* bark essential oil is compiled in Table 1. A total of 57 compounds were identified in *C. canadensis* bark oil accounting for 97.9% of the composition. The essential oil was dominated by fatty acid-derived compounds (76.0%), including 1-hexanol (23.3%), hexanoic acid (18.2%), (2E)-hexenoic acid (3.4%), oleamide (3.2%), and 1-docosanol (3.0%). n-Alkanes (10.2%), and aromatics (5.5%), were also present. Fatty acids and fatty acid-derived alcohols and aldehydes have sometimes been shown to be a feature of essential oils of the Fabaceae. For example, the bark essential oil of *Cassia bakeriana* from Brazil revealed 51.3% fatty acids, 23.2% aldehydes, and 11.1% alcohols [7]; although it did not contain any C6 compounds, the bark essential oil of *Inga laurina* from Brazil was composed of 46.8% fatty acids [8]; and the leaf essential oil of *Robinia pseudoacacia* growing in Poland was composed of 65.1% aliphatic alcohols [9].

Nakamura and Hatanaka have shown that C6 alcohols and aldehydes are bacteriostatic to several different strains of bacteria [10], but, in general, longer chain alcohols are more active [11, 12]. Huang and co-workers have demonstrated that medium-chain fatty acids as well as long-chain fatty acids exhibit antimicrobial activity; hexanoic acid was particularly active against *Candida albicans*, *Fusobacterium nucleatum*, and *Streptococcus mutans* [13].

Although the concentration of monoterpenoids was somewhat low, only 4.1%, it was possible to determine their enantiomeric distribution using chiral gas chromatography – mass spectrometry. α-Pinene was present as a racemic mixture, but limonene was present as the pure (+)-enantiomer. The (–)-enantiomers were the major stereoisomers for linalool (65%) and α-terpineol (70%). This, we believe, represents the first examination of the enantiomeric distribution of monoterpenoids in the Fabaceae.

4. Conclusions

The bark essential oil of *Cercis canadensis* was found to be rich in medium-chain and long-chain alcohols, aldehydes, and carboxylic acids, in particular C6 compounds. The presence of these compounds may account for the traditional use of *C. canadensis* bark by the Cherokee and other Native Americans. Although monoterpenoid concentrations were low, the chiral gas chromatographic analysis was able to discern the relative enantiomeric concentrations of α-pinene, limonene, linalool, and α-terpineol.

<table>
<thead>
<tr>
<th>RIb</th>
<th>Components</th>
<th>%</th>
<th>RIb</th>
<th>Components</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>799</td>
<td>Hexanal</td>
<td>0.9</td>
<td>1349</td>
<td>Eugenol</td>
<td>0.7</td>
</tr>
<tr>
<td>832</td>
<td>2-Methylbutanoic acid</td>
<td>1.0</td>
<td>1397</td>
<td>Methylheugenol</td>
<td>0.6</td>
</tr>
<tr>
<td>844</td>
<td>(3Z)-Hexenol</td>
<td>0.3</td>
<td>1418</td>
<td>β-Caryophyllene</td>
<td>0.7</td>
</tr>
<tr>
<td>849</td>
<td>(3E)-Hexenol</td>
<td>2.2</td>
<td>1446</td>
<td>Geranyl acetone</td>
<td>0.5</td>
</tr>
<tr>
<td>859</td>
<td>(2Z)-Hexenol</td>
<td>0.7</td>
<td>1508</td>
<td>Dicyclohexyl ketone</td>
<td>0.5</td>
</tr>
<tr>
<td>862</td>
<td>1-Hexanol</td>
<td>23.3</td>
<td>1580</td>
<td>Caryophyllene oxide</td>
<td>1.1</td>
</tr>
<tr>
<td>885</td>
<td>(4Z)-Hepten-2-ol</td>
<td>0.8</td>
<td>1600</td>
<td>n-Hexadecane</td>
<td>0.6</td>
</tr>
<tr>
<td>980</td>
<td>2-Heptanol</td>
<td>1.7</td>
<td>1607</td>
<td>1,10-di-epi-Cubanol</td>
<td>0.7</td>
</tr>
<tr>
<td>931</td>
<td>α-Pinene</td>
<td>0.2b</td>
<td>1654</td>
<td>α-Cadinol</td>
<td>0.9</td>
</tr>
<tr>
<td>967</td>
<td>1-Heptanol</td>
<td>0.6</td>
<td>1700</td>
<td>n-Heptadecane</td>
<td>0.8</td>
</tr>
<tr>
<td>975</td>
<td>Hexanoic acid</td>
<td>18.2</td>
<td>1793</td>
<td>1-Octadecane</td>
<td>0.5</td>
</tr>
<tr>
<td>977</td>
<td>1-Octen-3-ol</td>
<td>1.5</td>
<td>1800</td>
<td>n-Octadecane</td>
<td>0.6</td>
</tr>
<tr>
<td>1003</td>
<td>Octanal</td>
<td>0.4</td>
<td>1894</td>
<td>1-Nonadecane</td>
<td>0.7</td>
</tr>
<tr>
<td>1008</td>
<td>(2E)-Hexenoic acid</td>
<td>3.4</td>
<td>1900</td>
<td>n-Nonadecane</td>
<td>0.9</td>
</tr>
<tr>
<td>1028</td>
<td>Limonene</td>
<td>2.0b</td>
<td>1956</td>
<td>Palmitic acid</td>
<td>2.5</td>
</tr>
<tr>
<td>1032</td>
<td>Benzyl alcohol</td>
<td>1.3</td>
<td>1986</td>
<td>1-Eicosene</td>
<td>0.7</td>
</tr>
<tr>
<td>1042</td>
<td>Benzen acetaldehyde</td>
<td>1.0</td>
<td>2000</td>
<td>n-Eicosane</td>
<td>0.8</td>
</tr>
<tr>
<td>1069</td>
<td>1-Octanol</td>
<td>1.2</td>
<td>2100</td>
<td>n-Heneicosane</td>
<td>2.7</td>
</tr>
<tr>
<td>1083</td>
<td>α-Guaiaciol</td>
<td>0.3</td>
<td>2110</td>
<td>Methyl linoleate</td>
<td>1.2</td>
</tr>
<tr>
<td>1092</td>
<td>Unidentifieda</td>
<td>2.1</td>
<td>2200</td>
<td>n-Docosane</td>
<td>0.7</td>
</tr>
<tr>
<td>1099</td>
<td>Linalool</td>
<td>0.8e</td>
<td>2300</td>
<td>n-Tricosane</td>
<td>0.9</td>
</tr>
<tr>
<td>1104</td>
<td>Nonanol</td>
<td>1.8</td>
<td>2371</td>
<td>Oleamide</td>
<td>3.2</td>
</tr>
<tr>
<td>1111</td>
<td>2-Phenylethanol</td>
<td>0.6</td>
<td>2454</td>
<td>Docosanal</td>
<td>0.7</td>
</tr>
<tr>
<td>1139</td>
<td>(2E)-Nonenal</td>
<td>0.6</td>
<td>2517</td>
<td>1-Docosanol</td>
<td>3.0</td>
</tr>
<tr>
<td>1164</td>
<td>Octanoic acid</td>
<td>1.1</td>
<td>2600</td>
<td>n-Hexacosane</td>
<td>0.4</td>
</tr>
<tr>
<td>1194</td>
<td>α-Terpinol</td>
<td>0.7e</td>
<td>2700</td>
<td>n-Heptacosane</td>
<td>0.6</td>
</tr>
<tr>
<td>1205</td>
<td>Decanal</td>
<td>1.2</td>
<td>2806</td>
<td>(E,E,E)-Squalen</td>
<td>0.3</td>
</tr>
<tr>
<td>1230</td>
<td>2-Coumaranone</td>
<td>0.5</td>
<td>2900</td>
<td>n-Nonacosane</td>
<td>1.2</td>
</tr>
<tr>
<td>1249</td>
<td>Chavicol + Geraniol</td>
<td>0.8</td>
<td>Total Identified</td>
<td>97.8</td>
<td></td>
</tr>
</tbody>
</table>

*RI = “Retention Index”, determined with respect to a series of n-alkanes on a ZB-5 column. b 50% (+)-α-pinene / 50% (–)-α-pinene. c 100% (+)-limonene. a Unidentified: MS, m/e 196(2%), 128(2%), 101(23%), 99(44%), 83(52%), 71(48%), 55(100%), 45(38%), 43(74%), 41(34%).

d 35% (+)-linalool / 65% (–)-linalool. e 30% (+)-α-terpineol / 70% (–)-α-terpineol.*
5. References