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Identification of 5,6-dihydro-6-propyl-2H-pyran-2-one as the major volatile constituent in mesquite (*Prosopis*) flour

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ABSTRACT

Mesquite (*Prosopis spp.*) are woody leguminous plants that belong to the family Leguminosae and grow in arid and semiarid regions of America, Africa and Asia. *Prosopis spp.* produce indehiscent fruit (pods) that can be milled to yield flour that is sold commercially and is used in pastries and baked goods. The major volatile constituent of mesquite flour was identified as 5,6-dihydro-6-propyl-2H-pyran-2-one on the basis of its Kovats index and mass spectrum. Using δ-nonalactone as an internal standard its concentration was determined to be 59.75 ± 7.07 mg/kg (n = 3).

1. Introduction

Nitrogen fixing trees and shrubs of the genus *Prosopis* provide firewood, luxury quality timber for furniture and flooring, soil enrichment through nitrogen fixation on some of the world's most harsh arid ecosystems in North and South America, the Caribbean, Africa, the Middle East and the Indian subcontinent (Felker, 2009). Many *Prosopis* species produce yellow or reddish bean pods that are typically about 30% sucrose and 10–14% protein (Oduol, Felker, McKinley, & Meier, 1986). In what is today the southwestern US some Indian tribes, such as the Yuma and Cocopah, cultivated traditional foods in pre-Columbian times and wheat after the arrival of Europeans, but mesquite beans were one of the major, if not the most important, food sources of the desert Apache, Pima, Cahuilla, Maricopa, Yuma, Yavapai, Mohave, Walapi, and Hopi tribes (Bean & Saubel, 1972; Bell & Castetter, 1937; Felker, 2005). *Prosopis* pods were similarly important for indigenous peoples in the deserts of South America and India (Felker, 2005). The occurrence of significant percentages of trees with bitter pods in native populations of mesquites by indigenous peoples (Bean & Saubel, 1972) was confirmed in genetic improvement trials for *Prosopis alba* in Argentina (Felker et al., 2001) and Alban et al. (2002) for *Prosopis pallida* in Peru where trees were ranked for palatability and the most palatable trees cloned. Unfortunately, the nearly ubiquitous stands of introduced *Prosopis juliflora* in deserts of Kenya, Ethiopia, Somalia and Kenya are of the bitter type (Felker unpub obs). USDA organic, Kosher, gluten free mesocarp flour of the pods of *P. pallida* from Peru and *P. alba* from Argentina are being imported into the US for browning, flavour and aroma enhancement in gluten free and non-gluten free foods. The flavour volatiles of mesquite pods were investigated by Takeoka, Felker, Prokopiuk, and Dao (2008) who identified 121 constituents (14 of which were tentatively identified). The researchers were unable to characterise a major constituent that comprised 2.21%, 17.61% and 19.19% of the total volatiles in three mesquite pod samples. The goal of this research was to identify and quantify this major unknown mesquite pod constituent.

2. Materials and methods

2.1. Materials

Organic mesquite flour was obtained from Casa de Fruta (Hollister, CA). Sodium chloride and diethyl ether were purchased from Fisher Scientific (Pittsburgh, PA). Malonic acid, 2-hexenal and pyridine were from Sigma–Aldrich (Milwaukee, WI). δ-Nonalactone was received from Bedoukian research, Inc. (Danbury, CT). Anhydrous absolute ethanol (USP grade) was obtained from Pharmco (Brookfield, CT). Natural massoia lactone was purchased from A.M. Todd (Hamilton, OH). Diethyl ether was freshly distilled through a 60 cm long Pyrex column packed with glass helices and stored in the dark after addition of 1–2 mg/L of antioxidant 330 (1,3,5-trimethyl-2,4,6-tris[3,5-di-*tert*-butyl-4-hydroxybenzyl]benzene; Ethyl Corp., Richmond, VA).

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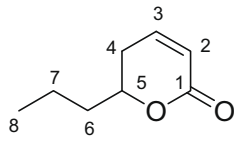

Fig. 1. Structure of 5,6-dihydro-6-propyl-2H-pyran-2-one.

2.2. Isolation of volatiles

Mesquite flour (30 g) was placed in a 1 L round-bottomed flask along with 54 g NaCl (previously heated at 150 °C for 4 h to remove volatiles) and 150 mL purified water (Milli-Q Plus, Millipore Corporation, Bedford, MA). Sixty microlitres of an internal standard solution of δ-nonalactone (49.6 mg dissolved in 100 mL absolute ethanol) was added to the flask. The flask was fitted with a Pyrex head to allow the sweep gas to enter the top of the flask (via a Teflon tube) and exit out of a side arm through a Tenax trap (ca. about 10 g of Tenax [Alltech Associates, Deerfield, IL], fitted with ball and socket joints). The system was purged with purified nitrogen (200–400 mL/min) for 2 min and immediately connected to an all-Teflon diaphragm pump (model UN726 FTP, KNF Neuberger, Inc., Trenton, NJ) that recirculated nitrogen around the loop (closed loop stripping) at 6 L/min for 3 h. The sample was continuously stirred during the sampling period with a magnetic stirrer. After sampling, the Tenax trap was removed and the volatiles eluted with 70 mL of freshly distilled ethyl ether prepared as described above. The extract was carefully concentrated to ca. 50 μL using a warm water bath (50 °C) and a Vigreux column.

2.3. Separation of volatiles

Extracts were analysed on an HP 6890 gas chromatograph equipped with a flame ionisation detector (FID). A DB-1 fused silica capillary column (60 × 0.32 mm i.d.; d_f = 0.25 μm; J&W Scientific, Folsom, CA) was employed. The DB-1 capillary column was programmed from 30 °C (4 min isothermal) to 200 °C (held for 15 min at final temperature) at 2 °C/min. The injector and detector temperatures were 180 °C and 280 °C, respectively. Helium carrier gas linear velocity was 38.5 cm/s (30 °C). Split injections (1:25) were used.

2.4. GC–MS analysis

Extracts were analysed on an Agilent Technologies 6890 N network gas chromatograph coupled to an Agilent Technologies 5973 Network MSD (Agilent Technologies, Palo Alto, CA). A 60 m × 0.25 mm (i.d.) DB-1 fused silica capillary column (d_f = 0.25 μm) was employed. The GC oven was programmed from 30 °C (4 min isothermal) to 200 °C at 2 °C/min (final hold = 25 min). The transfer line, ion source and quadrupole temperatures were 200, 170 and 130 °C, respectively. Helium was used as the carrier gas at a column head pressure of 22 psi. Split injections were utilised with an injector temperature of 180 °C. The mass spectrometer was operated in the electron impact mode at 70 eV, scanning the range of 35–350 m/z (4.51 scans/s) in a full scan mode acquisition.

2.5. Synthesis and purification of 5,6-dihydro-6-propyl-2H-pyran-2-one standard

5,6-Dihydro-6-propyl-2H-pyran-2-one was synthesized according to the method of May, Peterson, and Chang (1978). The compound was purified by preparative gas chromatography (Varian 3700 GC, Walnut Creek, CA) using a glass packed column (250 × 0.5 cm packed with 1% SF-96 on 120–140 mesh Chromosorb G).

2.6. Nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were obtained at 298 K from a sample dissolved in $CDCl_3$ with TMS as an internal standard on a Bruker model ARX400 spectrometer at a frequency of 100.62 MHz for carbon and 400.13 MHz for proton. One- and two-dimensional experiments were run for both nuclei. The number of attached protons for ^{13}C signals was determined from DEPT90 and DEPT135 assays.

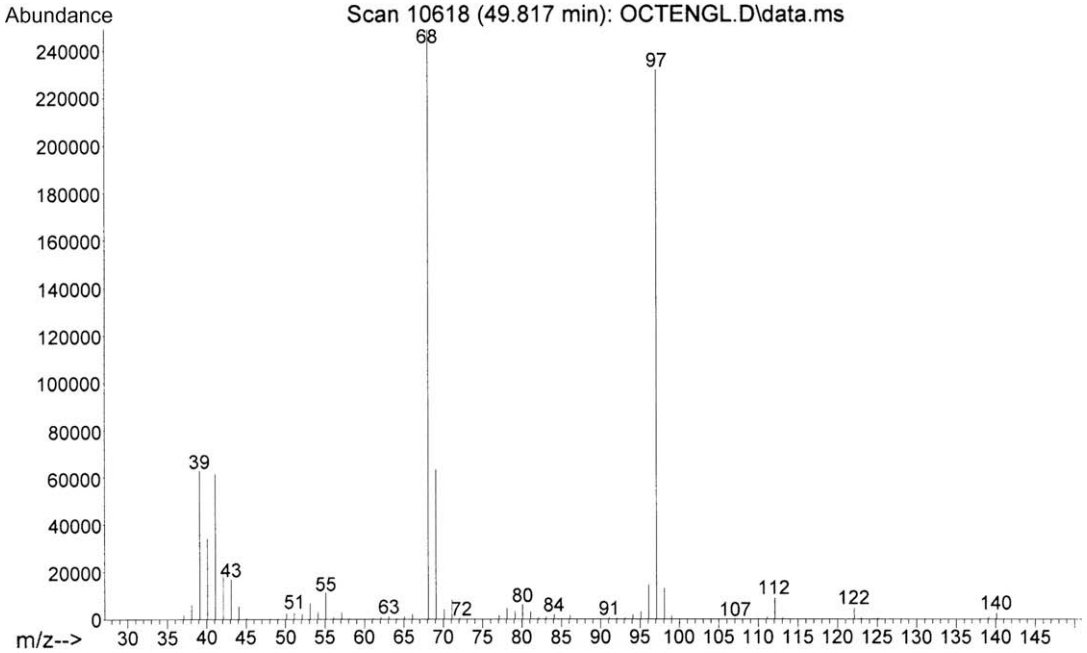

Fig. 2. Mass spectrum of 5,6-dihydro-6-propyl-2H-pyran-2-one.

3. Results and discussion

In a previous study on mesquite pod volatiles (Takeoka et al., 2008) a major constituent comprising between 2.21% and 19.19% of the total volatiles (in three different samples) was observed but was not identified. The unknown mesquite pod constituent had a mass spectrum similar to that of massoia lactone (5,6-dihydro-6-pentyl-2H-pyran-2-one) with major ions at 97 and 68 m/z (base peak). However, its Kovats index of 1210 was much lower than that of massoia lactone (I = 1423). It was suspected that the unknown was a homolog of massoia lactone with two less carbons since a small ion at 140 m/z was observed in the mass spectrum. On the basis of this evidence a standard of 5,6-dihydro-6-propyl-2H-pyran-2-one was synthesized according to the procedure of May et al. (1978). The structure of the standard is shown in Fig. 1 whilst the mass spectrum of the standard is displayed in Fig. 2. The synthesized standard had a similar mass spectrum (May et al., 1978; Urbach, Stark, & Nobuhara, 1972) and ^{1}H-NMR spectrum (Matsumoto & Nago, 1994) as previously reported in the literature. The ^{13}C-NMR spectrum (164.56 (C1), 144.93 (C2), 121.53 (C3), 29.43 (C4), 77.76 (C5), 36.96 (C6), 18.11 (C7), 13.81 (C8)) of the standard was very similar to that of massoia lactone for the ring carbons though the side chain carbons differed as expected (Fournier, Kocienski, & Pons, 2004; Ishikawa, Amaike, Itoh, Warita, & Kitahara, 2003). This compound has been rarely reported in natural products though it has been evaluated as a food flavouring constituent (May et al., 1978; Nobuhara, 1968). 5,6-Dihydro-6-propyl-2H-pyran-2-one was identified as a trace constituent in air-cured Burley tobacco by Fujimori, Kasuga, Matsushita, Kaneko, and Noguchi (1976). Matsumoto and Nago (1994) identified 5,6-dihydro-6-propyl-2H-pyran-2-one as the major volatile produced by *Lasiodiplodia theobromae* GK-1 (a strain of filamentous fungi isolated from the surface of the fruit of coconut palms) grown on potato-dextrose-agar (PDA) media. These researchers described the compound's odour as coconut-like mixed with a peppermint aroma. Ricardo et al. (2004) identified 5,6-dihydro-6-propyl-2H-pyran-2-one in the roots and stems of *Cryptocarya ashersoniana* seedlings. The compound exhibited moderate antifungal activity (with a minimum inhibitory concentration (MIC) of 8 μg compared to standard fungicidal compounds, nystatin (MIC = 0.5 μg) and myconazol (MIC = 0.5 μg)) when assayed using bioautography with phytopathogenic fungi, *Cladosporium cladosporioides* and *C. sphaerospermum* (Ricardo et al., 2004).

Volatiles were isolated from mesquite flour using a closed loop dynamic headspace sampling method (Buttery, Teranishi, & Ling, 1987). Gas chromatographic analyses revealed that the main constituent was an unknown that constituted about 14.6% of the total area. The identity of the unknown was confirmed as 5,6-dihydro-6-propyl-2H-pyran-2-one since its Kovats index (I = 1210) and mass spectrum closely matched that of the synthesized standard. δ-Nonalactone was employed as an internal standard to determine the concentration of 5,6-dihydro-6-propyl-2H-pyran-2-one in mesquite flour. The concentration of 5,6-dihydro-6-propyl-2H-pyran-2-one in mesquite flour was determined to be 59.75 ± 7.07 mg/kg (n = 3). Though the odour threshold of this compound has not been reported the odour threshold of the related δ-octalactone is 0.5 mg/kg. It might be expected that 5,6-dihydro-6-propyl-2H-pyran-2-one has an odour threshold of a similar magnitude as δ-octalactone so it probably contributes to the odour of mesquite flour. Since mesquite flour is typically used in pastries and baked goods there is the question of how much 5,6-dihydro-6-propyl-2H-pyran-2-one is lost during baking. It has been observed that lactones averaged about a 25% loss during baking of crackers (Gary Reineccius, personal communication) so we might expect a similar level of loss with this unsaturated lactone as the result of heat treatment.

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