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Solubility of the Sesquiterpene Alcohol Patchoulol in Supercritical Carbon Dioxide

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The solubility of the sesquiterpene alcohol patchoulol in supercritical carbon dioxide was measured at pressures (P) ranging from 10.0 MPa to 25.0 MPa and temperatures (T) of 40.0 and 50.0 °C using a simple microsampling-type apparatus with a 100.5 μL sample loop to remove aliquots for off-line analysis. The system was first validated using vanillin with off-line spectrophotometric analysis, then utilized for patchoulol measurements with off-line GC−MS analysis. The measured solubility of patchoulol in supercritical CO_2 ranged from mole fractions of 0.43×10^{-3} at 10.0 MPa and 50.0 °C to mole fractions of 9.4×10^{-3} at 25.0 MPa and 40.0 °C.

Introduction

The sesquiterpene patchoulol (patchouli alcohol, CAS Registry Number 5986-55-0) is a white, crystalline solid at room temperature that was first isolated and described in 1869.[1] The chemical structure of patchoulol is shown in Figure 1. The tricyclo[5.3.1.0]undecane carbon skeleton of patchoulol is somewhat unusual and has made its chemical synthesis the subject of study by several groups over the years.[1−6] Patchoulol typically represents 30 % to 40 % of the total mass of the dozens of compounds in patchouli oil, an essential oil commonly obtained by steam distillation of the leaves of *Pogostemon cablin* (Benth.) which is used extensively in the perfume and fragrance industry.[7] In fact, *Pogostemon cablin* has been cultivated for patchouli oil production for over 100 years.

It has long been held that patchouli oil possesses favorable perfume, antifungal, antidepressant, and insect repellent properties,[8−10] especially in Asian countries and India. For example, the essential oil has historically been used to repel moths from clothing. Patchouli oil and its individual components, including patchoulol, are contributors to traditional Chinese medicines which are currently being examined for use as fungicides and for the inhibition of fungal infections.[11] Recently, Zhu et al. reported that both patchouli oil and purified patchoulol itself were toxic and repellent against Formosan subterranean termites, with topical patchoulol noted to cause substantial damage to the termite exoskeleton.[12] They concluded that the antitermite effect of patchouli oil was due to the patchoulol component.

In addition to steam distillation to isolate patchouli oil, Huang et al. recently found that supercritical carbon dioxide extraction methods could be used to collect a volatile oil from *Pogostemon cablin* which was 48.8 % patchoulol. This work indicates that supercritical fluid extraction processes might be advantageous for the isolation of purified patchoulol, either from natural products or from synthetic mixtures.[3,4,13]

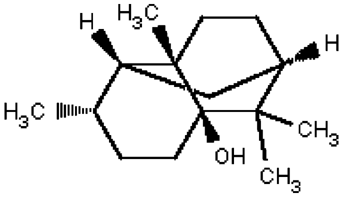

Figure 1. Structure of patchoulol (patchouli alcohol).

Processes using supercritical fluids are of great research interest for the synthesis, extraction, analysis, and comminution of materials, and some commercial applications have arisen from these studies.[14−17] This interest is due in part to the “green” nature of some supercritical fluids compared to the organic solvents they are proposed to replace and in part to the tunable nature of the solvent properties of supercritical fluids.[18,19] The most widely used supercritical fluid has been carbon dioxide, due to its low cost, availability, reasonably low critical temperature and pressure, low toxicity, and adequate solvent properties for lipophilic solutes. Effective utilization of supercritical carbon dioxide for extraction or processing is facilitated by measurements of solubilities in supercritical carbon dioxide.[14,15,20,21] In the present work, a sampling loop method was used to determine the solubility of patchoulol in supercritical carbon dioxide; to the author’s knowledge, these are the only solubility data reported for patchoulol in carbon dioxide.

Experimental Section

Materials. Solid, crystalline patchoulol (CAS 5986-55-0, shown in Figure 1) was a kind gift from International Flavors and Fragrances, Inc. (New York, NY). Pure carbon dioxide (Coleman grade, 99.99 %) was obtained from General Air Service & Supply (Denver, CO), and vanillin (99.5 %) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Absolute ethanol was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY), and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). The reported purity levels of the vanillin and patchoulol solutes were verified by thin-layer chromatography, and the compounds were used as received.

Apparatus and Procedure. A schematic of the experimental apparatus is shown in Figure 2. It is a microsampling system with a high-pressure sample loop and valve system

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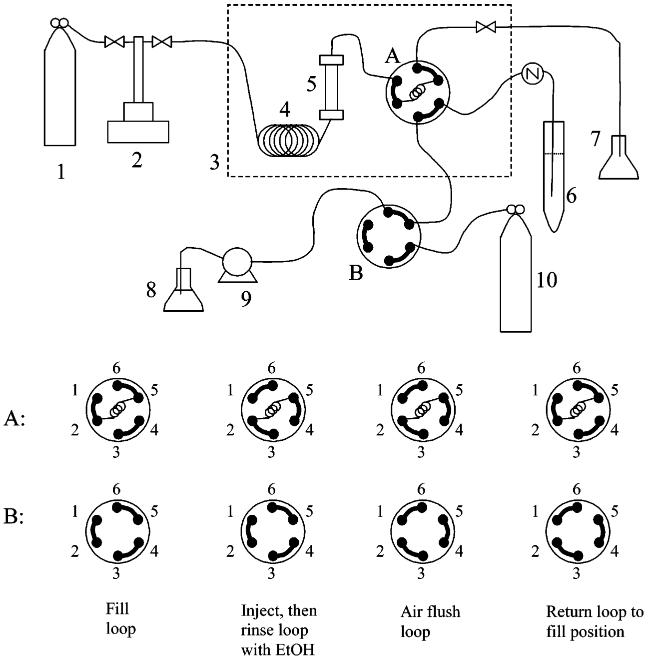

Figure 2. Schematic diagram of the apparatus used: 1, CO$_2$ cylinder with dip tube; 2, syringe pump; 3, thermostated water bath; 4, preheater coil; 5, saturator; 6, off-line sample collection tube; 7, waste collection flask; 8, ethanol reservoir; 9, HPLC pump; 10, pressurized air; A, six-port valve with sample loop; B, six-port valve.

adapted from the system described by McHugh and Paulaitis[22] and is similar to the apparatus used in many previous reports in which the static sampling strategy has been used.[14,23] Such systems utilize a sample loop to remove small portions of a saturated supercritical fluid solution for analysis. To test the present system, it was used first to determine the solubility of vanillin in carbon dioxide for comparison to published values and validation of the system,[24] and then it was used to determine the solubility of patchoulol in carbon dioxide. A high-pressure syringe pump (Isco model 260D, Teledyne Isco, Inc., Lincoln, NE) was used to pressurize the carbon dioxide (P = (10.0 MPa to 25.0 MPa), ±0.5 %), which was directed through a coil of 1/16 in. stainless steel tubing as a preheater, contained within a recirculating, temperature-controlled water bath (VWR Scientific Model 1131, West Chester, PA), and then directed through a 7.5 mL stainless steel extraction vessel as a saturator (Keystone Scientific, Bellefonte, PA) packed with solute (vanillin or patchoulol) and 3 mm glass beads, with glass wool used to trap the solid solute in the center of the vessel. The pressures delivered by the syringe pump at each set point were measured using a mechanical Bourdon tube-type gauge rated at ±0.25 % accuracy (Helicoid Gage, ACCO, Bridgeport, CT), and the precision of the set pressures was verified to be ±0.5 %. The fittings on the inlet and outlet of the saturator vessel contained sintered stainless steel frits to act as filters and prevent undissolved solute from being swept into the sample loop. The preheater, saturator, and sampling loop were all contained within the circulating bath, and the continuously circulating water was maintained at a constant temperature (T = 40.0 °C or 50.0 °C, calibrated with a Hg thermometer and controlled to within ±0.5 °C). Flow through the saturator was stopped for 1 h at each set temperature and pressure, with pressure maintained from the syringe pump, to give sufficient time to approach saturation conditions.[20] Effluent from the saturator was then directed for 1 min at 0.5 mL·min through a nominally 100 μL sample loop attached to a six-port, two-way valve (Rheodyne Model A7000, Rohnert Park, CA, referred to here as valve A) using an adjustable valve (fill position) and then vented to waste. When filled with saturated solution, the valve was turned to the inject position which allowed the loop to depressurize through a needle valve into a preweighed collection vial outside of the temperature-controlled bath into which 2 mL of ethanol solvent was added to trap the solute, and then the loop was flushed with an additional 3 mL of ethanol into the collection vial using an HPLC pump (Waters Model 501, Milford, MA) connected to a second Rheodyne six-port, two-way valve (valve B, rinse position), on which only three of the ports were utilized. The total amount of ethanol solvent used to collect the solute was determined gravimetrically (±0.0001 g). Before switching valve A back to the fill position for the next sample collection, valve B was switched (air-flush position) and the lines and sample loop were flushed with compressed air to remove ethanol otherwise trapped within. Then valve B was switched back to the rinse position with flow from the HPLC pump turned off. Multiple samples were collected at each pressure and temperature combination.

Sample Loop Volume Calibration. The volume of the sample loop (100.5 ± 2 μL) was determined by measuring the amount of dilution of a vanillin solution of known concentration in ethanol. Briefly, the sample loop was loaded by syringe with the standard solution, then directed into a preweighed collection tube using the HPLC pump and ethanol. The amount of dilution was determined spectrophotometrically using a Beckman DU600 spectrophotometer (Fullerton, CA) at 279 nm, a relative absorption maxima for vanillin, and a 1 cm quartz cell. The final volume was determined gravimetrically. For determination of concentrations, a standard curve was prepared for absorption at 279 nm of vanillin solutions of 0.3 μM, 1.5 μM, 3 μM, 15 μM, 30 μM, and 150 μM in ethanol, and a linear fit was obtained with a correlation coefficient >0.99. A wavelength scan of one of our vanillin standards in ethanol gave a relative maximum absorbance at 279 nm, in agreement with published data,[25,26] and a molar absorptivity on the order of 10^4 L·mol·cm at this wavelength.

Quantitation of Carbon Dioxide. The carbon dioxide solution densities were estimated to be the same as those for pure carbon dioxide, which has been shown to be accurate for solutions with a mole fraction <1 %.[23, 27] To verify this approximation, we measured the gas volume of carbon dioxide (±0.5 mL) discharged from the loop at each pressure and temperature, with and without saturation with patchoulol solute. The gas volumes (ranging from about 20 mL to 65 mL) were identical at each pressure and temperature combination for pure carbon dioxide and for carbon dioxide saturated with patchoulol, supporting the prior assertions and indicating the validity of using pure carbon dioxide densities to quantitate the solvent. To determine the molar amount of solvent contained within the known loop volume, the carbon dioxide densities at each pressure and temperature used were determined using an equation of state;[28] to accomplish this, the online version of software from the U.S. National Institute of Standards and Technology was used (http://webbook.nist.gov/cgi/fluid.cgi?ID=C124389&Action=Page) which allows calculations of the density, and other thermophysical properties, of carbon dioxide at specified temperatures and pressures.

Quantitation of Solutes. The solute collected in ethanol was quantitated by spectrophotometry (for vanillin) or GC−MS (for patchoulol), and the values presented are the means of three or more repetitions. For vanillin, which was used for comparison with previous solubility data and to verify the currently used

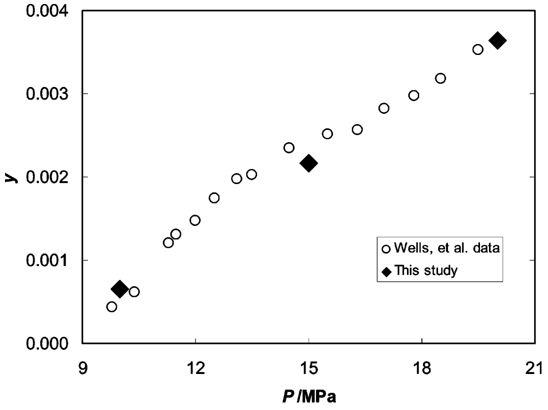

Figure 3. Solubility of vanillin in carbon dioxide at 45.0 °C as mole fraction (y) to validate the microsampling apparatus used in this study against previous results from Wells et al.[24]

apparatus and method, triplicate runs were collected at $T = 45.0$ °C and $P = 10.0$ MPa, 15.0 MPa, and 20.0 MPa, and the collected samples were first diluted 1:20 with additional ethanol and then analyzed by spectrophotometry at 279 nm with a 1 cm quartz cell and quantitated by comparison to a standard curve made using vanillin and ethanol at dilutions of 0.3 μM to 150 μM as described above. Patchoulol samples were collected at $T = 40.0$ °C and 50.0 °C and $P = 10.0$ MPa, 15.0 MPa, 20.0 MPa, and 25.0 MPa, and the resultant ethanol solutions were analyzed by GC−MS. A wavelength scan of a patchoulol standard in ethanol gave a relative maximum absorbance at 309 nm, but the solution was nearly transparent, with a molar absorptivity of only about 1.5 L·mol·cm at this wavelength. A GC−MS method was therefore adapted from previous work by Zhu et al.[12] In the present work, the collected samples were pure patchoulol of unknown concentrations in ethanol, meaning that the quantitation of patchoulol did not require separation from a complex mixture, so the chromatography was greatly simplified compared to previous reports.[11,13] Briefly, patchoulol was resolved on a 30 m DB-23 column, with 0.25 μm film thickness, using a pulsed splitless injection of 1 μL aliquots using an Agilent 6890 GC−MS with an autosampler. The inlet temperature was 250 °C. The pulse pressure was 32 psi for 0.5 min, and then the run pressure was 19.58 psi. The column temperature started at 150 °C and was increased at 30°/min to a maximum of 250 °C. The retention time of patchoulol under these conditions was approximately 2.7 min, and the peak shape was narrow and symmetrical. For detection, the chemical ionization (CI) mode was used, with a solvent delay of 2.05 min. The $[M - OH]^+$ ion was abundantly formed by CI, so this m/z value (205.1) was monitored for detection. To verify the results, the $[M - H]^+$ ion at $m/z = 221.1$ was also monitored. External standards of patchoulol in ethanol from 6.25 μM to 6.25 mM were used to generate a linear standard curve ($r^2 = 1.00$) which was used to quantitate the patchoulol concentrations in the samples.

Results and Discussion

To validate the solubility measurement system used for the present study, the solubilities of vanillin in supercritical CO_2 were first determined experimentally using the microsampling apparatus, and the data were compared to previously published results. As shown in Figure 3, the solubilities of vanillin at $T = 45.0$ °C and $P = 10.0$ MPa, 15.0 MPa, and 20.0 MPa determined using our system are in close agreement with the data from Wells et al.[24] and with the data in recent work by Skerget et al. (data not shown).[29] This indicates that the apparatus used in the present study is appropriate for supercritical CO_2 solubility determinations of patchoulol, which like vanillin is a lipophilic, crystalline solid.

Table 1. Solubility of Patchoulol in Supercritical CO_2: Temperature *T*, Pressure *P*, Supercritical CO_2 Density[a] ρ, Mole Fraction *y*, and Concentration *C*

T/°C	P/MPa	ρCO_2/mol·L^{-1}	y ($\times 10^3$) ± SEM	C/g·L^{-1} ± SEM
40.0	10.0	14.28	1.8 ± 0.3	5.7 ± 0.9
40.0	15.0	17.73	4.0 ± 0.9	16 ± 4
40.0	20.0	19.08	6.0 ± 1.2	25 ± 5
40.0	25.0	19.98	9.4 ± 1.3	42 ± 6
50.0	10.0	8.73	0.43 ± 0.01	0.84 ± 0.03
50.0	15.0	15.9	3.1 ± 0.1	10.8 ± 0.3
50.0	20.0	17.82	4.8 ± 0.2	18.8 ± 0.7
50.0	25.0	18.96	7.7 ± 0.7	33 ± 3

[a] Supercritical carbon dioxide density from the NIST Webbook calculation reference.[28]

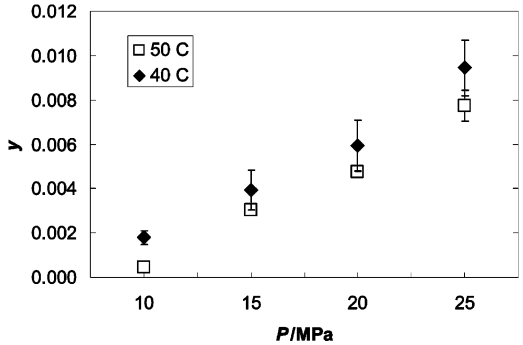

Figure 4. Solubilities of patchoulol in carbon dioxide at 40.0 and 50.0 °C presented as mole fraction (y), based on the mean of three measurements with the standard error of the mean shown.

The solubilities of patchoulol in supercritical CO_2 are presented in Table 1 and Figure 4. The solubility of patchoulol in supercritical CO_2 ranged from mole fractions (y) of 0.43×10^{-3} at 10.0 MPa and 50.0 °C to y values of 9.4×10^{-3} at 25.0 MPa and 40.0 °C. As shown in Figure 4, the mole fraction (y) of patchoulol in supercritical CO_2 increased with increasing pressure at constant temperature and decreased with increasing temperature at constant pressure. Each point is the average of three measurements ± the standard error of the mean (SEM). The variability of the solubility data was presented as the SEM to indicate how well the determined mean solubility value represents the actual solubility at those conditions. Potential sources of experimental uncertainty in the solubility measurements include possible deposition of solid patchoulol within the decompression region between the sampling loop and the collection vial and possible loss of postexpansion patchoulol particulates that could have blown through the ethanol solvent in the collection vial.

Conclusions

The solubilities of patchoulol in supercritical CO_2 were determined at 40.0 °C and 50.0 °C in the pressure range of 10.0 MPa to 25.0 MPa. The observed solubilities ranged from mole fractions (y) of ($0.43 \times 10^{-3} \pm 0.01 \times 10^{-3}$) to ($9.4 \times 10^{-3} \pm 1.3 \times 10^{-3}$). Under these experimental conditions, the patchoulol solute was a crystalline solid and the mole fraction in supercritical CO_2 increased with increases in pressure at constant temperature and decreased with increases in temperature at constant pressure. The results support the idea that supercritical carbon dioxide may be useful for the processing of patchouli oil and the preparation of purified patchoulol.

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