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Original Research Article

Isolation of (-)-Patchouli Alcohol from Patchouli Oil by Fractional Distillation and Crystallization

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Abstract

Purpose: *To establish a new and efficient method for the isolation of (-)-patchouli alcohol (PA) from patchouli oil (PO) .*
Methods: *PO, obtained from commercial source, was separated into four fractions (A, B, C and raffinate) using fractional distillation according to pre-set reflux ratio in vacuum. PA was crystallized from fraction C (containing more than 80 % PA by weight) by cooling and centrifugation. Finally, PA was further purified by suction filtration. Characterization of PA was performed by melting point (MP), infrared spectroscopy (IR), ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).*
Results: *The total yield of PA in this procedure reached 52.9 %. The structure of PA was obtained based on data from ^{1}H-NMR, ^{13}C-NMR and MS analysis with the aid of literature data for authenticated samples.*
Conclusion: *Fractional distillation combined with crystallization can be successfully applied to the isolation of PA from PO in solvent-free conditions.*

Keywords: *Isolation, Patchouli alcohol, Patchouli oil, Fractional distillation, Crystallization*

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INTRODUCTION

(-)-Patchouli alcohol (PA, the chemical structure is shown in Figure 1), is a tricyclic sesquiterpene with a distinct odor of patchouli-like earthy and camphor. Nowadays, PA has been widely used in chemicals, flavors, and perfumery industry. It is used as a fragrance ingredient with a consumption of 0.1 – 1.0 metric ton per year [1].

PA is the major constituent of patchouli oil (PO), and there are many methods such as steam distillation [2], supercritical extraction [2] and molecular distillation [3, 4] used in practice for the separation and purification of PA from PO. Nevertheless, all of these methods fail to obtain high-purity PA.

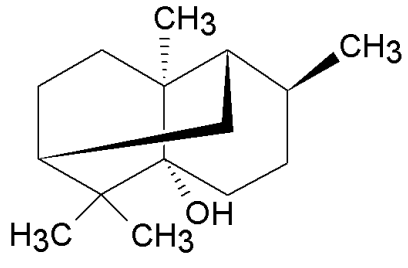

Figure 1: Chemical structure of (-)-Patchouli alcohol

In addition, methods of silicagel column [5], host-guest inclusion [6] and distillation combining with crystallization [7] had also been developed and successfully applied to the isolation of PA. However, the recovery rates of PA obtained by these methods are lower than 40 %, and these methods have many shortcomings, including large energy and/or chemical reagent consumption, solvent residue, low yield, instability and small-scale production.

Therefore, the application of PA has been hampered due to lack of economical and practical methods of manufacture of PA. Consequently, the aim of this study is to develop a large-scale manufacturing method for the isolation of PA from PO in solvent-free conditions. Fractional distillation is a highly efficient and solvent-free separation process which depends on the differences of components' boiling points in vacuum. Crystallization is a separation process that depends on the differences in components' freezing point and degree of saturation. Hence, a method combining fractional distillation with crystallization should be feasible and suitable for the isolation of PA from PO.

EXPERIMENTAL

Material

Patchouli oil (Lot.120212) was purchased from Guangzhou Baihua Flavours and Fragrances Company Ltd. (Guangzhou, China).

Apparatus

Separation of PA was carried out with a fractionating tower consisting of a temperature control heating jacket, a round-bottom flask (1000 mL), a fractionating column (150 × 25 mm inner Diameter) filled with stainless steel Dixon ring packing (3 × 3 mm inner Diameter), an intelligent thermostat and a reflux condenser. The reflux ratio (reflux flow (L) to distillate flow (D), defined by American Society for Testing and Materials) was controllable and expressed as L/D value. The fractionating tower was operated *in vacuum* using a PC 101 NT chemistry pumping unit (Vacuubrand GmbH & Co. KG, Wertheim, Germany). Distillates were centrifuged with a 2-16PK table-top refrigerated centrifuge (Sigma Zentrifugen GmbH, Osterode, Germany).

The distillates were determined by as gas chromatography-mass spectrometer (GC-MS) system consisting of an Agilent 6890 gas chromatography instrument, a 5973 mass spectrometer and an Agilent ChemStation software (Agilent, Palo Alto, USA).

Fractional distillation and crystallization procedure

The PO was placed in the round-bottom flask of fractionating tower, and the pressure of this system was kept at 6 kPa. PO was heated at 200.0 °C until refluxing, and then maintained at 195.0 °C for 3 h. Subsequently, the concentration of PA in distillates was analyzed by GC-MS every 30 min while temperature was keep at 215.0 °C. The process flow diagram for PA from PO is shown in Figure 2. According to respective distilled-off temperature, the distillates were designated fraction A (containing no PA), fraction B (containing PA < 80 % by weight), fraction C (containing PA ≥ 80% by weight) and raffinate. Afterwards, fraction C was cool at 15 °C for 10 min, and then centrifuged at 3000 rpm for 10 min (15 °C). PA crystals were obtained after filtration.

Gas chromatography (GC)-mass spectrometry (MS) analysis

The distillates diluted with n-hexane (1:6) were analyzed by GC-MS. Chromatographic separation was carried on a 5% phenyl methyl siloxane HP-5MS capillary column (30 m × 0.25 mm inner Diameter, 0.25 μm film). The oven temperature was set initially at 60 °C, followed by a gradient of 3 °C /min up to 140 °C (held for 1 min), and then programmed to 160 °C at 1 °C /min (held for 1 min), finally, the temperature was up to 230 °C at 10 °C /min (held for 1 min). Split injection (1 μL) was conducted with a split ratio of 60:1 and helium was used as carrier gas with 1.0 mL/min flow-rate. The spectrometer was set in electron-impact (EI) mode, the ionization energy was 70 eV, the scan range was 50–400 amu and the scan rate was 0.34 s per scan. The inlet and the ionization source temperatures were 250 and 280 °C, respectively. Identification of the compounds was based on data from Wiley/NBS Registry of Mass Spectral Data (V.5.0) and National Institute of Standards and Technology (NIST) MS Search (2011, V.2.0).

Structure identification

Melting point (MP) was determined by a B-545 melting point in capillary tubes (Büchi, Switzerland). Optical rotation was measured by a Model 341 Polarimeter (PerkinElmer, USA) and $[\alpha]_D$ value was given in units of 10^{-1} deg cm2 g^{-1}. IR spectra were determined with an Impact 410 spectrometer (Nicolet Company, USA). The spectra were recorded in a transmittance mode

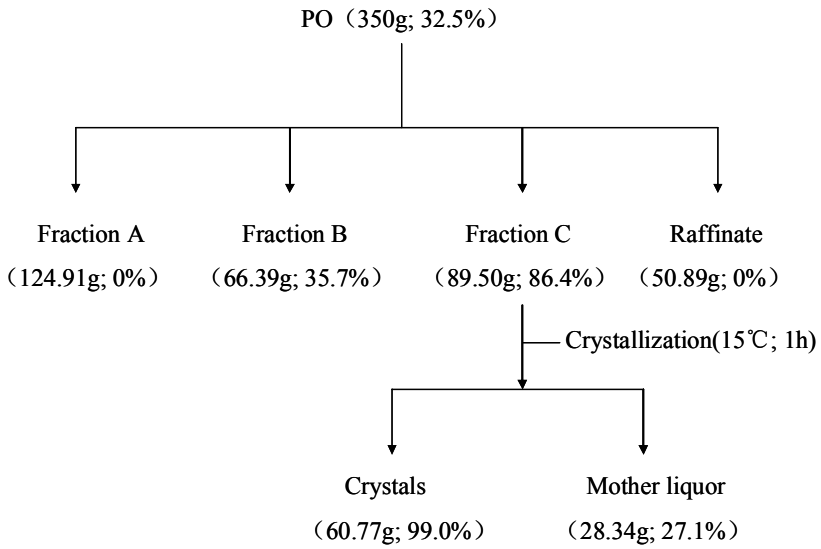

Figure 2: Separation and purification of PA. The yield (weight of patchouli oil, fraction, crystals and mother liquor; concentration of PA)

from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz)spectra were recorded on JNM EX-400 nuclear magnetic resonance spectrometer (JEOL Ltd., Japan), $CDCl_3$ was used as a solvent and tetramethylsilane as an internal reference. Chemical shifts (δ) were expressed in ppm shift. Mass spectra were recorded on MAT TSQ 7000 mass spectrometer using direct inlet (EI) mode (Finngan Company, USA).

RESULTS

Separation and purification of PA

The weight and PA content of all fractions described above are shown in Figure 2. By fractional distillation, PO (350.00g, 32.5%) was separated into fraction A (124.91g, 0.0%), fraction B (66.39g, 35.7%), fraction C (89.50g, 86.4%) and raffinate (50.89g, 0%). Then fraction C was crystallized to obtain PA crystals (60.77g) with a concentration of 99.0 %. PA was white crystalline, and its physicochemical properties and spectra data of IR, EI-MS, ^{1}H NMR and ^{13}C NMR were fairly in accord with experimental results reported in literature [5].

The total yield of PA in this procedure reached 52.9 %, and total separation time was about 7.5 h without any chemical reagents consumption. In contrast, the yield from column chromatography method for manufacture of PA was < 10 % [5]. From the foregoing results, fractional distillation coupled with crystallization is an environment-friendly method capable of large-scale production to isolate and purify PA from PO.

Chemical composition of all fractions

To investigate the process of isolation and purification of PA from PO, the chemical compositions of PO distillates were analyzed by GC - MS. The total ion current (TIC) chromatogram of PO, fraction (A, B, C) and PA was shown in Figure 3. Components with lower boiling points than that of PA was distillated prior to PA and divided into fraction A and fraction B (Figure 3B, 3C), then fraction C consisting mainly of PA was obtained (Figure 3D). At last, as Figure 3E shown, the production of PA was high purity.

Yield of PA from fraction C at different reflux ratios

As shown in Table 1, yields of PA in fraction C and separation time were measured according to different reflux ratios.

DISCUSSION

The feasibility of this process

In the present study, fractional distillation combined with crystallization was successfully applied to the isolation of PA from PO. PO is a commercial flavor, with a mature production technology and market supply. There are some standards for the quality control of PO, and Chinese Pharmacopoeia prescribes that the content of PA in commercial PO should not to be less than 26% [8]. Therefore, the source of PO is stable and adequate, which is beneficial to the large-scale production of PA from patchouli oil by fractional distillation and crystallization.

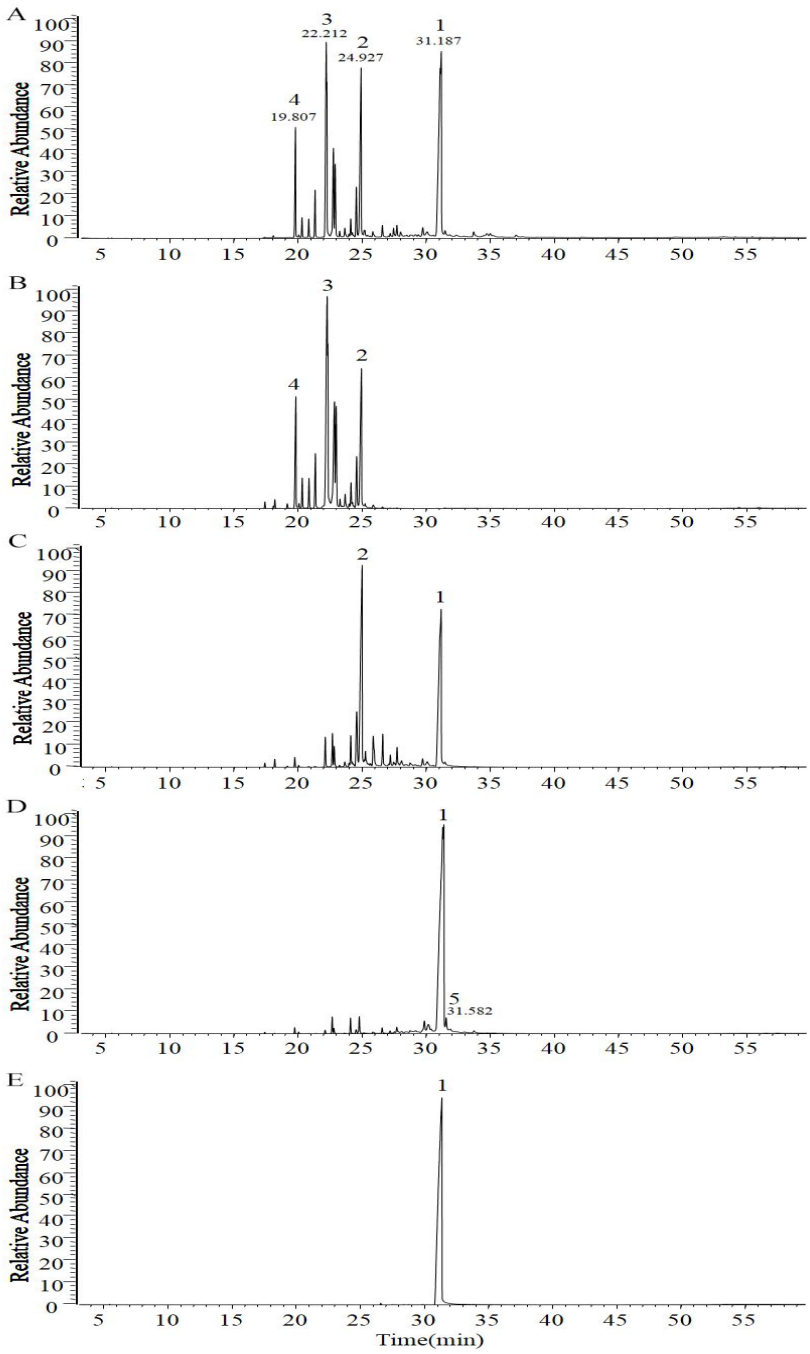

Figure 3: TIC chromatograms of PO, fraction A, fraction B, fraction C and purified PA were shown in A, B, C, D and E, respectively. And peak 1, 2, 3, 4, 5 were PA, δ-guaiene, α-longipinene, β-patchoulene and longipinocarrone, respectively.

Table 1: Yield of PA in fraction C and separation time with different reflux ratios

No.	Reflux ratio control	Yield of PA from fraction C (%)	Separation time (h)
1	3:1[a], 3:1[b], 3:1[c]	39.7	6.0
2	3:1, 5:1, 5:1	40.8	8.0
3	5:1, 3:1, 7:1	67.9	7.5
4	7:1, 4:1, 9:1	68.1	9.5

[a] Reflux ratio as fraction A distilled off; [b] reflux ratio as fraction B distilled off;
[c] the reflux ratio as fraction C distilled off. The yield of PA from fraction C (%) is the ratio of the amount of PA in fraction C to the amount of of PA in patchouli oil in percentage.

Physical basis for the separation and purification procedure

As shown in the total ion current (TIC) chromatogram of PO (Figure 3A), boiling points of most components of PO such as β-patchoulene, α-longipinene and δ-guaiene are rather lower than that of PA. Fractional distillation is a high efficient and solvent-free separation process, which depends on the differences of components' boiling points in a high vacuum. Consequently, components with lower boiling points than that of PA can been distillated prior to PA, and divided into fraction A and fraction B (Figure 3B, 3C), then fraction C consisted mainly of PA was obtained (Figure 3D).

However, as TIC chromatogram of fraction C shows, there are some components such as longipinocarrone which have similar molecular weights and boiling points as PA are difficult to be removed by fractional distillation. However, these components are quite different from PA in their freezing points. Crystallization is a separating process depending on the difference of components' freezing points and degree of saturation. Furthermore, the concentration of PA in fraction C attained the saturated state at 15 °C but not for others. Accordingly, PA (Figure 3E) was purified by crystallization at 15 °C from fraction C.

CONCLUSION

Natural PA can be isolated from PO under solvent-free conditions using fractional distillation and crystallization technique. The yield of PA, coupled with its purity and the separation time, indicate that this procedure was a more effective and environmentally friendly method, as compared with other methods of synthesis and separation. Furthermore, the aromatic odors of all distillates from PO by this procedure will not be broken and could also be available as fragrance ingredients. In the light of the physical basis for separation of PA, fractional distillation in combination with crystallization can be expected to provide an economical and practical method for the separation and purification of bioactive components from volatile oils.

Authors contribution

Su ZQ and Wu XL equally contributed to this work. All authors conceived and designed the study, collected and analysed the data as well as prepared and approved the final manuscript for publication.

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