Notes

Efficient Purification of Labile and Volatile Reaction Products by Near-Critical Fluid Chromatography (NCFC)

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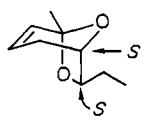
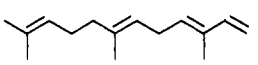
Introduction

Despite the numerous options available to synthetic organic chemists for the purification of crude reaction products by chromatographic means, notably flash column chromatography, preparative gas chromatography (GC), and high-performance liquid chromatography (HPLC), problems are often encountered due to decomposition on the column or losses through volatilization. With preparative HPLC, fraction collection is trivial; however, in dealing with volatile substances, appreciable losses occur due to solvent removal. While preparative GC handles volatile compounds adequately, collected fractions often contain decomposition products and contaminants due to column "bleed". Preparative GC has also a disadvantage in its low capacity. As shown in this work, these main problems of HPLC and GC, encountered typically with volatile and unstable compounds, can be minimized through the use of a near-critical mobile phase with a conventional HPLC column.

To demonstrate the preparative advantages of near-critical fluid chromatography (NCFC), we have chosen two cases of synthesized mammalian pheromones, (*S,S*)-3,4-dehydro-*exo*-brevicomin and (*E,E*)-α-farnesene.



(*S,S*)-3,4-dehydro-*exo*-brevicomin



(*E,E*)-α-farnesene

Racemic dehydrobrevicomin has been shown effective as a component of the male mouse multipurpose pheromone.[1] (*E,E*)-α-Farnesene (along with (*E*)-β-farnesene) appears to act as the male mouse dominance signal.[2] In our biological experiments with these and related substances, it has been necessary to obtain exceptionally pure samples of synthetic materials. For both substances, purification by preparative GC or HPLC was found much less suitable than by near-critical fluid chromatography due to the loss of solutes during solvent evaporation of HPLC fractions or decomposition of solutes in the hot GC injector and detector.

During the last several years, supercritical fluids such as carbon dioxide and nitrous oxide have been increasingly utilized in efficient extractions[3] and analytical chromatographic separations.[4,5] It is widely appreciated that supercritical fluids often possess liquidlike solubilization power, while they are intermediate between gases and liquids with respect to solute diffusivity and viscosity, resulting in highly efficient mass transport processes. Yet, except for some model reactions carried out in near-critical media,[6,7] applications in organic chemistry have been rare. Investigating the efficiency of such media for purification purposes has thus been logical.

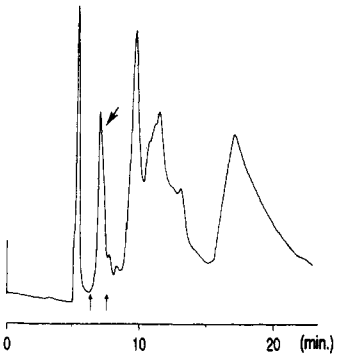


Figure 1. Near-critical fluid separation of crude (*S,S*)-3,4-dehydro-*exo*-brevicomin in CO_2 at subcritical conditions: 25 °C, 85 atm, Phenomenex Ultromex 5 C_{18} packed column 25 cm × 4.6 mm, i.d., UV detection at 215 nm, injection volume 10 μL. The two marked lines indicate the fraction collected. Arrow indicates the peak of interest (the same designation is used in all the following figures).

Results and Discussion

Our previous attempts to purify the synthetic crude (*S,S*)-3,4-dehydro-*exo*-brevicomin[8] and (*E,E*)-α-farnesene,[9] as well as a variety of other labile or volatile synthetic products, by preparative GC or HPLC were consistently less successful than near-critical fluid chromatography, described here, due to the losses of volatile solutes during solvent evaporation of HPLC fractions or decomposition of labile compounds during the GC process. As seen in Figure 1, the separation of (*S,S*)-3,4-dehydro-*exo*-brevicomin from other major components of a crude reaction mixture has been accomplished with subcritical carbon dioxide as the mobile phase at 25 °C. Figure 2 shows the capillary GC comparison of crude product (A) with the purified substance (B) according to Figure 1. The major peak (Figure 2B) was confirmed to be 3,4-dehydro-*exo*-brevicomin by gas chromatography/mass spectrometry.

Similarly, the crude product of (*E,E*)-α-farnesene[9] was subjected to fractionation by near-critical fluid chromatography (Figure 3). Once again, Figure 4 (parts A and

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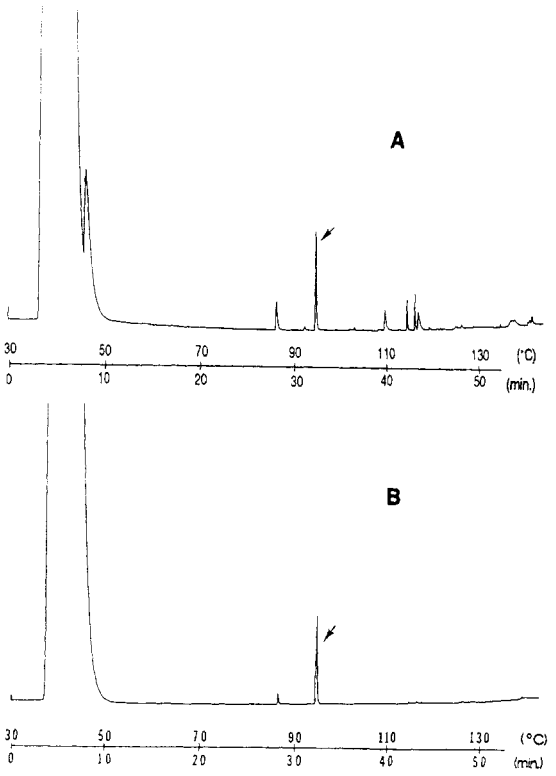

Figure 2. Capillary GC analysis of (*S,S*)-3,4-dehydro-*exo*-brevicomin: (A) before NCFC purification; (B) after NCFC purification. GC conditions: Ucon 50-HB-2000 coated capillary, 60 m × 0.25 mm, i.d., temperature program 30–160 °C, at 2 °C/min, splitless injection of 0.2 μL of 0.2% solution in hexane.

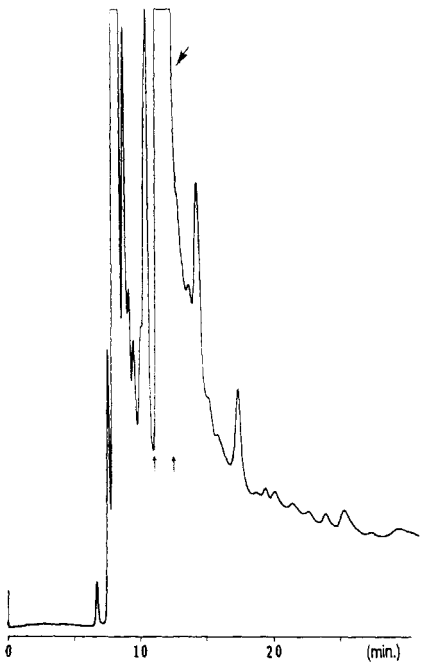

Figure 3. Near-critical fluid separation of crude (*E,E*)-α-farnesene. Conditions as in Figure 1.

B) contrasts purities of the original material and the appropriate recovered fraction. As with the dehydro-*exo*-brevicomin purification, we were able to recover (*E,E*)-α-farnesene easily with minimal losses and in a pure state.

Figure 5 shows an additional comparison of two different fractionation techniques: preparative GC (A) and near-critical fluid chromatography (B). Both chromatograms were obtained using subcritical carbon dioxide as the mobile phase. The column where the crude (*E,E*)-α-

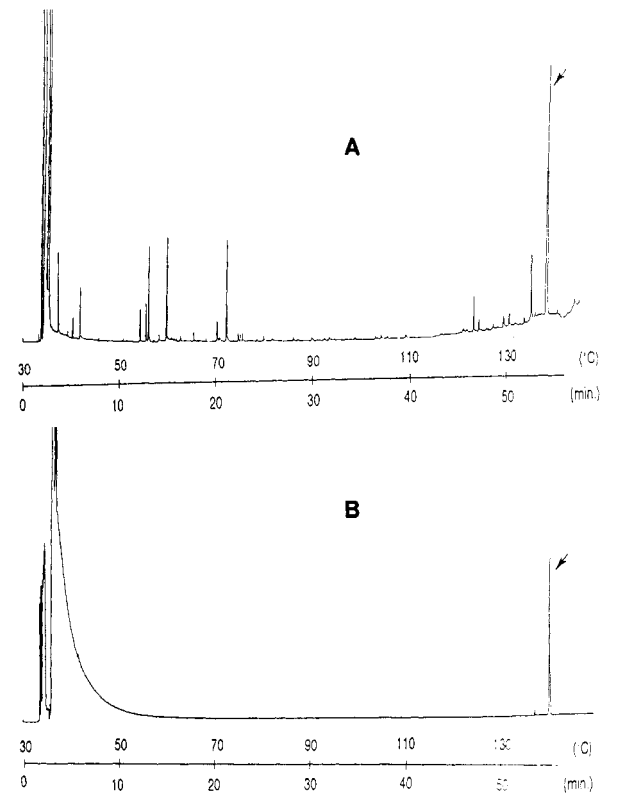

Figure 4. Capillary GC analysis of α-farnesene: (A) before NCFC purification; (B) after NCFC purification. GC conditions as in Figure 2.

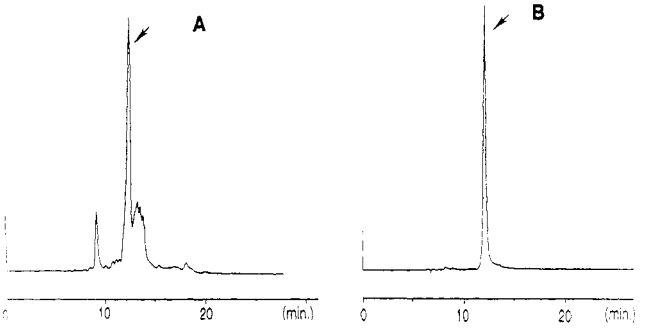

Figure 5. Near-critical fluid chromatography analysis of α-farnesene purified by: (A) preparative GC; (B) near-critical fluid chromatography. Injection volume, 0.2 μL, other conditions as in Figure 1.

farnesene was intially separated is now shown in the role of an "analytical column". This labile compound is rechromatographed as a single peak following the near-critical fluid fractionation (B); however, after GC preparative separation at 160 °C with detector temperature at 250 °C, several additional peaks were observed (A). Since (*E,E*)-α-farnesene is a labile compound, its decomposition during GC fractionation is understandable. In the vicinity of their critical points, compressed gases exhibit certain desirable properties that make them uniquely suitable for efficient extractions and chromatography.[10] Whereas most analytical applications utilize the media above the critical point (supercritical fluid chromatography), the critical isotherm can be passed to the subcritical region without a large discontinuity in transport properties of these fluids.[11] Consequently, we use the term "near-critical

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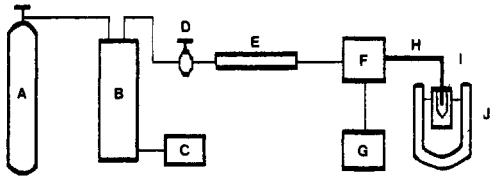

Figure 6. Schematic diagram of the NCFC purification system. (A) CO_2 tank; (B) pump; (C) microcomputer; (D) injector valve; (E) HPLC column; (F) UV detector; (G) chart recorder; (H) capillary restrictor; (I) sample microvial; (J) liquid nitrogen container–Dewar flask.

fluid" in this paper to emphasize this choice, although the use of lower temperatures appears logical in purification of labile substances.

Although purification of (*S,S*)-3,4-dehydro-*exo*-brevicomin and (*E,E*)-α-farnesene by near-critical fluid chromatography have been described here as examples, we have applied similar procedures successfully to other volatile and thermolabile compounds.

Experimental Section

The near-critical fluid chromatography system, constructed in this laboratory from various HPLC and GC hardware components, consisted of a 250-mL Varian 8500 syringe pump (Walnut Creek, CA), modified for pressure control through a microcomputer as published by Van Lenten and Rothman,[12] a UV-absorbance detector with a 3.5-μL high-pressure flow cell (Isco, Lincoln, NE), a conventional HPLC column, and an injection valve with a 10-μL loop (Rheodyne, Cotati, CA), as shown in Figure 6. Phenomenex Ultromex 5 C_{18} 25 cm × 4.6 mm, i.d., or Water Nova-Pak C_{18}, 30 cm × 3.9 mm, i.d., columns were utilized. A 50 cm × 50 μm, i.d., fused silica capillary was attached to the detector outlet as a pneumatic restrictor.

(*S,S*)-3,4-Dehydro-*exo*-brevicomin[8] and (*E,E*)-α-farnesene[9] were prepared as described.

Synthetic products and the fractions recovered by near-critical fluid chromatography were analyzed by capillary GC (a modified Varian 1400 gas chromatograph) and peak identities were ascertained by gas chromatography/mass spectrometry (GC/MS) using a Hewlett-Packard 5981 instrument with Incos data system (Finnigan MAT, San Jose, CA). The capillary column used (60 m × 0.25 mm, i.d.) was coated statically using Ucon 50-HB-2000, with benzyltriphenylphosphonium chloride added as a column deactivation agent.[13] Preparative GC of (*E,E*)-α-farnesene was carried out with a Varian 3700 gas chromatograph, using a thermal conductivity detector and a packed column (3 m × 1.2 mm, i.d.) with 6% SE-30 stationary phase on a 100–120 mesh Supelco port (Supelco, Inc., Bellefonte, PA).

The fractions from the near-critical fluid separation were collected directly into a 3-mL sample microvial immersed in liquid nitrogen (Figure 6). Typically, 5–10-μL aliquots of crude synthetic products were injected into the packed column. While pressurized carbon dioxide was used as the mobile phase, the column head pressure was held at 85 atm, and the separations were carried out at 25 °C. The fractions were collected by inserting the capillary restrictor end through a small hole in the cap of the vial. When half the volume of the collection vial became filled with frozen column effluent, the vial was removed from the liquid nitrogen. By slightly loosening the cap, carbon dioxide was allowed to escape at room temperature, while the solute remained in the vial. After collection, the vial was completely sealed with an intact cap and stored in the freezer before analysis or biological tests were performed.

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Structures of Autoxidation Products of 2-*tert*-Butyl-4-methoxyphenol in Aqueous Alkaline Solution

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2-*tert*-Butyl-4-methoxyphenol (**1**) is a major component of BHA, an extensively used antioxidant. Oxidation of **1** with various oxidants afforded 3,3′-di-*tert*-butyl-2,2′-dihydroxy-5,5′-dimethoxybiphenyl (**2**), presumably formed by coupling of the aryloxy radicals derived from **1**.[2-5] Kurechi et al. reported isolation of **2** and its isomer 2′,3-di-*tert*-butyl-2-hydroxy-4′,5-dimethoxydiphenyl ether as the products of ultraviolet-irradiated autoxidation of **1** in ethanol.[6,7] Oxidation of **1** in an aqueous alkaline solution with atmospheric oxygen was also studied and the solid that precipitated in the course of oxidation was characterized as **2**.[8] In this note we will describe the isolation and structure determination of four components other than **2** from the alkaline autoxidation mixture of **1**.

Results and Discussion

Autoxidation of 2-*tert*-butyl-4-methoxyphenol (**1**) was carried out in a 0.5 M NaOH solution. The solution of **1** was vigorously stirred to attain intimate contact with atmospheric oxygen. As oxidation proceeded, the slightly colored solution turned into a dark red mixture from which a powder precipitated gradually. After 1 week, all of **1** had been consumed and the mixture was filtered to give the crude dimeric product **2** in 46% yield. The filtrate was acidified by concentrated HCl and extracted with ether. Concentration of the ether layer induced precipitation of a white solid, which was collected by filtration. The solid was almost homogeneous, consisting of one main component (**3**, $C_{19}H_{26}O_5$; yield 1.8%). The mother liquor of **3** was evaporated to give a dark brown oil, which was chromatographed over Al_2O_3. Elution with benzene gave yellow crystals in 5.4% yield. SiO_2 TLC analysis indicated that the crystals were composed of two components, **4a** and **4b**, in the ratio of ca. 6:4. Isolation of each compound was attained by recrystallization from hexane and SiO_2 column chromatography.

Compound **4a**, mp 168–171 °C, showed a molecular ion peak $(C_{18}H_{22}O_3)^+$ in the high resolution mass spectrum. Three carbonyl stretching bands at 1720, 1680, and 1630 cm^{-1} in the IR spectrum accounted for the three oxygen atoms of the molecular formula. The ^1H NMR spectrum of **4a** taken in $CDCl_3$ exhibited five singlets, indicating the presence of two *tert*-butyl groups (δ 1.29 and 1.32), one

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