

# Determination of the Enantiomeric Composition of 2-Methoxy-3-(1'-methylpropyl)pyrazine from Galbanum Oil Using Achiral and Chiral Lanthanide Shift Reagents

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<sup>1</sup>H NMR examination of 3-methoxy-2-(1'-methylpropyl)pyrazine 1-oxide (2) derived from 2-methoxy-3-(1'-methylpropyl)pyrazine (1), with use of achiral and chiral lanthanide shift reagents, showed that the enantiomeric composition of 1 isolated from Galbanum oil is 100% (+). Signal assignment of enantiomers was achieved by comparison of <sup>1</sup>H NMR spectra of four kinds of 2 derived from natural 1 and synthetic (S)-(+)-, (R)-(-)-, and (±)-1. The odor thresholds of (S)-(+)-, (R)-(-)-, and (±)-1 were 0.10, 0.01, and 0.04 ppb, respectively.

Galbanum oils is widely used in perfumery. Bramwell et al. (1969) have isolated from Galbanum oil 2-methoxy-3-(1'-methylpropyl)pyrazine (1), which possesses an intense odor. The odor differences between the two optical isomers of carvone (Leitereg et al., 1971; Russell and Hills, 1971; Friedman and Miller, 1971), patchouli alcohol (Naf et al., 1981), nootkatone (Haring et al., 1972), and other fragrance and flavor materials (Demole et al., 1982; Gautier et al., 1987) have been recognized. Chiral lanthanide NMR shift reagents are extensively used in the detection of enantiomeric purity (Schurig, 1985). In this work, a chiral lanthanide NMR shift reagent, Eu(hfc)<sub>3</sub>, was utilized for the enantiomeric composition of 1 isolated from Galbanum oil.

## EXPERIMENTAL SECTION

**Instrumentation.** Specific rotations  $[\alpha]_D^{20}$  were measured in a 0.1-dm tube on a Otsuka Electronics Model PM-101 automatic digital polarimeter. The infrared (IR) spectra (neat liquids) were taken on a Hitachi 260-10 instrument. <sup>1</sup>H NMR spectra were recorded on a AM-400 Bruker instrument with CDCl<sub>3</sub> as the solvent. Chemical shifts are expressed (δ scale) downfield from TMS internal standard. GC analyses were carried out on a Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (coated with Carbowax 20M). The column temperatures were programmed from 80 to 200 °C at a rate of 3 °C/min. GC/MS was measured at 70 eV on a Hitachi M-80B spectrometer. The gas chromatographic column and oven conditions were as described for the Hewlett-Packard gas chromatograph. The preparative TLC was carried out on 20 × 20 cm glass plates precoated with silica gel 60 F<sub>254</sub> (0.5 mm, Merck).

**Materials.** All starting chemicals were obtained from reliable commercial sources and used without further purification.

**Fractionation of Galbanum Oil.** Commercially obtained Galbanum oil (1 kg) was distilled on a Vigreux column and then a Perkin-Elmer spinning-band distillation system (Model 251 Auto Annular Still). Fractions were collected at 77–84 °C (10 mm), and thus 9.6 g of oil was obtained. The distillate separated by column chromatography (eluting with various ratios of ethyl acetate-*n*-hexane mixtures), and preparative TLC (eluting with 1:9 acetone-*n*-hexane) gave crude (56%) 2-methoxy-3-(1'-methylpropyl)pyrazine (1): *R<sub>f</sub>* 0.69–0.75; 34 mg.

**N-Oxidation of 1.** To crude 1 (34 mg) were added 30% hydrogen peroxide (0.12 g) and acetic acid (0.06 g). The reaction mixture was stirred and heated at 80 °C for 18 h. This solution was neutralized with 30% sodium hydroxide solution. The organic layer was extracted with ether. The extract was washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated, and subsequent preparative TLC (eluting with 6:1 dichloromethane-

ethyl acetate) of the product gave 3-methoxy-2-(1'-methylpropyl)pyrazine 1-oxide (2): *R<sub>f</sub>* 0.18; 11 mg.

**Synthesis of (S)-(+)- and (R)-(-)-2-Methoxy-3-(1'-methylpropyl)pyrazine [(S)-(+)- and (R)-(-)-1].** (S)-(+)- and (R)-(-)-1 were synthesized from (2S,3S)-(+)-isoleucine and (2S,3R)-(+)-isoleucine, respectively (Murray and Whitfield, 1975). (S)-(+)-1:  $[\alpha]_D^{20} +19.7^\circ$  (c 0.152, CHCl<sub>3</sub>). (R)-(-)-1:  $[\alpha]_D^{20} -17.4^\circ$  (c 0.288, CHCl<sub>3</sub>). The enantiomeric compositions of (S)-(+)- and (R)-(-)-1 were determined by <sup>1</sup>H NMR of (S)-(+)- and (R)-(-)-2, which were derived from (S)-(+)- and (R)-(-)-1, with Eu(hfc)<sub>3</sub>, and were 98 and 90%, respectively.

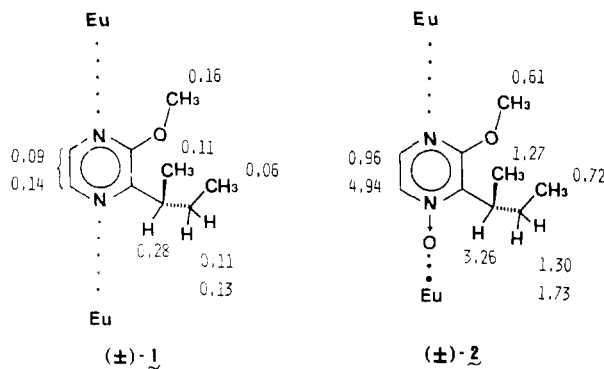
**Oxidation of (S)-(+)- and (R)-(-)-2-Methoxy-3-(1'-methylpropyl)pyrazine [(S)-(+)- and (R)-(-)-1].** A solution of (S)-(+)-1 (0.2 g, 1 mmol) and 30% H<sub>2</sub>O<sub>2</sub> (0.8 g, 7 mmol) dissolved in acetic acid (0.45 g) was heated at 75–80 °C for 18 h. The reaction mixture was neutralized by adding 30% sodium hydroxide aqueous solution and extracted with ether. The ether layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated, and subsequent preparative TLC (eluting with 6:1 dichloromethane-ethyl acetate) of the product gave (S)-(+)-3-methoxy-2-(1'-methylpropyl)pyrazine 1-oxide ((S)-(+)-2): *R<sub>f</sub>* 0.18; yield 0.19 g (87%);  $[\alpha]_D^{20} +1.6^\circ$  (c 0.860, CHCl<sub>3</sub>).

The enantiomeric composition was determined by <sup>1</sup>H NMR with use of Eu(hfc)<sub>3</sub> and was 98%: IR (neat) 2960, 1590, 1520, 1460, 1405, 1360, 1270, 1150, 1110 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>) δ 0.84 (3 H, t, *J* = 7.4 Hz, H<sub>3</sub>-3'), 1.31 (3 H, d, *J* = 7.1 Hz, H<sub>3</sub>C-1'), 1.72 (1 H, ddq, *J* = 13.3, 7.4 and 7.4 Hz, H<sub>a</sub>-2'), 1.93 (1 H ddq, *J* = 13.3, 7.8, and 7.4 Hz, H<sub>b</sub>-2'), 3.80 (1 H, m, H-1'), 3.98 (3 H, s, OCH<sub>3</sub>), 7.76 (1 H, d, *J* = 4.2 Hz, H-6), 7.79 (1 H, d, *J* = 4.2 Hz, H-5); MS, *m/z* 182 (M<sup>+</sup>, 3%), 167 (13), 166 (10), 165 (100), 140 (62), 137 (26), 135 (11), 133 (10), 123 (26), 118 (10), 93 (13). Anal. Calcd for C<sub>9</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: 182.1055. Found: 182.1046.

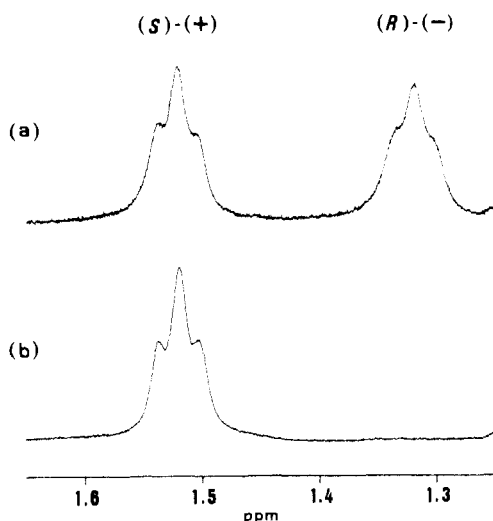
(R)-(-)-2 was prepared from (R)-(-)-1 by the same method to prepare (S)-(+)-2. (R)-(-)-2:  $[\alpha]_D^{20} -1.4^\circ$  (c 0.702, CHCl<sub>3</sub>). The enantiomeric composition was determined as above and was 90%. Anal. Calcd for C<sub>9</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>: 182.1055. Found: 182.1069.

**Synthesis of (±)-2-Methoxy-3-(1'-methylpropyl)pyrazine [(±)-1].** (±)-(1'-Methylpropyl)pyrazine was prepared from the parent pyrazine by alkylation with ethyl methyl ketone (Bramwell et al., 1971). This compound has been chlorinated with sulfonyl chloride and subsequently allowed to react with the sodium methoxide to obtain (±)-1 (Bramwell et al., 1972).

**Sensory Evaluation.** Threshold values for the pyrazines were determined by the "2/5 test" (selecting the same two out of five samples) employed by Amoore (1970). The panelists (seven males) ranged in age from approximately 22 to 29 years of age. They had extensive experience and proven reliability in odor judgements. The odor characteristics of the pyrazines were evaluated at about 100 times higher concentrations than the thresholds. The sessions were conducted midmorning of every day.



**Figure 1.** Chemical shifts induced by  $\text{Eu(DPM)}_3$  ( $\Delta\delta$ ) in  $\text{CDCl}_3$  for  $(\pm)$ -1 and  $(\pm)$ -2. Molar ratios of  $\text{Eu(DPM)}_3$  to both  $(\pm)$ -1 and  $(\pm)$ -2 are 0.28. The positive values indicate downfield shift (ppm).



**Figure 2.**  $\text{H}_3$ - $3'$  signals of 3-methoxy-2-(1'-methylpropyl)pyrazine 1-oxide (**2**) derived from (a) synthetic  $(\pm)$ -1 and (b) **1** in Galbanum oil, in the presence of  $\text{Eu(hfc)}_3$ . Molar ratios of  $\text{Eu(hfc)}_3$  to **2** are 0.28.

## RESULTS AND DISCUSSION

2-Methoxy-3-(1'-methylpropyl)pyrazine (**1**) which possesses an intense and characteristic odor of the Galbanum oil, has been isolated from the Galbanum oil as shown in literature (Bramwell et al., 1969).  $(S)$ -(+)-,  $(R)$ -(-)-, and  $(\pm)$ -**1** were synthesized according to the general methods described by Murray and Whitfield (1975) or Bramwell et al. (1971, 1972).

Chiral lanthanide shift reagents were found to cause large enantiomeric shift differences in the NMR spectrum of several chiral compounds (Schurig, 1985).  $\text{Eu(hfc)}_3$  at the lanthanide to substrate molar ratio ( $[\text{L}]/[\text{S}]$ ) 0.29–2.22 was found to cause little enantiomeric shift differences ( $\Delta\Delta\delta$ ) in the NMR spectrum of  $(\pm)$ -**1**.  $(\pm)$ -**1** was converted to the pyrazine  $N$ -oxide  $(\pm)$ -**2**, in order to observe nonequivalent spectra for enantiomeric **2** in the presence of  $\text{Eu(hfc)}_3$ . By high-resolution MS, the formula of  $(\pm)$ -**2** was calculated as  $\text{C}_9\text{H}_{14}\text{N}_2\text{O}_2$ . The absorption band at  $1270\text{ cm}^{-1}$  in the IR spectrum revealed an  $N$ -oxide function in  $(\pm)$ -**2**. An  $\text{M}^+ - 17$  ( $m/z$  165) peak was observed in the mass spectrum of  $(\pm)$ -**2** as a base peak. It is known that an  $\text{M}^+ - 17$  ion peak appears in the mass spectra of aromatic  $N$ -oxides carrying an alkyl group  $\alpha$  to the  $N$ -O group (Uchimaru et al., 1971). Achiral lanthanide shifts  $[\text{Eu(DPM)}_3]$  were also helpful in elucidating the substituted position. The chemical shifts induced by  $\text{Eu(DPM)}_3$  ( $\Delta\delta$ ) in  $\text{CDCl}_3$  for  $(\pm)$ -**1** and  $(\pm)$ -

**2** are summarized in Figure 1. The  $\Delta\delta$  values for  $(\pm)$ -**2** are greater than those for  $(\pm)$ -**1**. The most obvious changes for  $(\pm)$ -**2** involve the low-field movement of the H-6 (4.94 ppm) and H-1' (3.26 ppm). We interpret these results as signifying major binding of the lanthanide atom at the N-1 oxygen in  $(\pm)$ -**2** as shown in Figure 1. Then,  $(\pm)$ -**2** was suggested to be 3-methoxy-2-(1'-methylpropyl)pyrazine 1-oxide.

Figure 2 shows the  $\text{H}_3$ - $3'$  signals of 3-methoxy-2-(1'-methylpropyl)pyrazine 1-oxide (**2**) derived from (a) synthetic  $(\pm)$ -**1** and (b) **1** in Galbanum oil, in the presence of  $\text{Eu(hfc)}_3$ . It was found that the  $\text{H}_3$ - $3'$  signal of  $(S)$ -(+)-**2** appears at the lower field. It may be concluded that the enantiomeric composition of **1** from the Galbanum oil was found to be 100%  $(S)$ -(+).

Sensory evaluations showed the odor threshold of synthetic  $(\pm)$ -**1** to be 0.04 ppb in water. For  $(S)$ -(+)- and  $(R)$ -(-)-**1**, the respective values found were 0.10 and 0.01 ppb. It is noteworthy that a threshold value of the naturally occurring  $(S)$ -(+)-**1** is higher than that of the non-natural  $(R)$ -(-)-**1**. Murray et al. (1970) reported the threshold value of **1** in water, which was prepared from isoleucine, to be 0.001 ppb. The enantiomeric evidence of this compound has not been mentioned.  $(S)$ -(+)-,  $(R)$ -(-)-, and  $(\pm)$ -**1** have a burdocklike, green and earthy odor. Qualitatively, there is no significant difference between the organoleptic properties of  $(S)$ -(+)-,  $(R)$ -(-)-, and  $(\pm)$ -**1**, while the  $(R)$ -(-)-**1** appears to have a somewhat more burdocklike aroma than its antipode,  $(\pm)$ -**1**, and behaves just like a mixture of both forms.

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**Registry No.** ( $\pm$ )-1, 123625-20-7; (S)-(+)-1, 124151-13-9; (R)-(-)-1, 124151-14-0; ( $\pm$ )-2, 124151-15-1; (S)-(+)-2, 124070-50-4; (R)-(-)-2, 124070-51-5; ( $\pm$ )-(1-methylpropyl)pyrazine, 124070-52-6; pyrazine, 290-37-9; ethyl methyl ketone, 78-93-3; ( $\pm$ )-2-chloro-3-(1-methylpropyl)pyrazine, 124070-53-7; L-isoleucine, 73-32-5; D-isoleucine, 319-78-8.

## Variation of Flavor Components on Leaf Surfaces of Tobacco Genotypes Differing in Trichome Density

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Three populations of tobacco-doubled haploids (DH) were evaluated to characterize the relationship between density of secreting glandular trichomes (SGT) and levels (micrograms per square centimeter of leaf surface) of three trichome exudate constituents and to identify DH lines with altered concentrations of *cis*-abienol, sucrose esters containing  $\beta$ -methylvaleric acid (BMVSE), and  $\alpha$ - and  $\beta$ -duvatriediols (DVT). In the two populations in which some DH lines had few or no SGT, density scores for SGT were correlated positively with concentrations of *cis*-abienol, BMVSE, and DVT. All plants in the third population of DH lines had SGT, but SGT scores and concentrations of exudate constituents were not correlated. Several DH lines with DVT levels 2–3 times greater than the parent genotypes were identified. The results suggest that in some populations selection for glandular trichome density could alter levels of flavor and aroma constituents on the leaf surface.

Trichomes on leaves of many tobacco (*Nicotiana tabacum* L.) genotypes terminate in multicellular glands that are known to have biosynthetic capability (Keene and Wagner, 1985). Depending on the genotype, the exudate or secretion product of glandular trichomes may contain chemical compounds that influence insect and disease resistances and directly, or via their degradation products, affect flavor and aroma of tobacco products (Severson et al., 1985b). Recent evidence (Kandra and Wagner, 1987; Keene and Wagner, 1985; Wagner, 1988) suggests that the biosynthesis of sucrose esters (6-*O*-Acetyl-2,3,4-tri-*O*-acyl- $\alpha$ -D-glucopyranosyl- $\beta$ -D-fructofuranoside),  $\alpha$ - and  $\beta$ -4,8,13-duvatriene-1,3-diols (DVT), and possibly the labdane, *cis*-abienol (12(*Z*)-labda-12,14-dien-8 $\alpha$ -ol) occurs in head cells of secreting glandular trichomes (SGT). Non-secreting glandular trichomes (NSGT) are present on leaves of some genotypes. These trichomes are morphologically similar to SGT except they lack exudate.

Relatively large quantities of sucrose esters and *cis*-abienol are typically found on the leaf surface of Oriental tobacco genotypes, but they are not present in significant quantities in the other two major types, burley and flue-cured tobaccos, used in cigarette manufacture (Severson et al., 1985b). Enzell (1976), Kallianos (1976), and Smeeton (1987) suggested that these compounds impart flavor and aroma properties characteristic of the Turkish or Oriental types of tobacco. DVT's are present in

all three of these tobacco types with SGT, but DVT concentrations vary among tobacco types.

The inheritance of trichome exudate constituents and other trichome traits has been fairly well documented. A single gene influences the presence of *cis*-abienol in tobacco (Coussirat et al., 1983/84). Similarly, the production of sucrose esters containing  $\beta$ -methylvaleric acid (BMVSE) is controlled by a single gene (Gwynn et al., 1985). Coussirat et al. (1983/84) suggested that two genes control the presence of DVT's in tobacco. Other genetic studies have also shown that a single gene affects the secreting capability of glandular trichomes (Nielsen et al., 1982) and that the density of glandular trichomes is controlled by three genes (Johnson et al., 1988).

Breeding efforts designed to alter the concentration of *cis*-abienol, BMVSE, and DVT could be based on selection for density of SGT, if these traits are related. This would eliminate many chemical analyses and permit selection prior to flowering and seed production and would facilitate breeding progress. However, there is little direct evidence suggesting a relationship between the density of glandular trichomes and the level of these leaf surface compounds. Increasing the density of SGT in tobacco may increase the concentration of chemical constituents associated with these structures. Therefore, the main objective of this study was to determine the extent of the relationship between density of SGT and the levels of DVT's, *cis*-abienol, and BMVSE on the leaf surface. An additional objective was to characterize the extent of variation for leaf surface compounds in populations of doubled haploids developed from crosses among geno-

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