New Pyridine Derivatives and Basic Components in Spearmint Oil (*Mentha gentilis* f. *cardiaca*) and Peppermint Oil (*Mentha piperita*)

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The basic fraction of spearmint Midwest Scotch oil (spearmint MWS, *Mentha gentilis* f. *cardiaca*) was analyzed by fused silica capillary gas chromatography and mass spectrometric techniques. A total of 38 nitrogen-containing components including 11 new pyridine derivatives, 2-isopropyl-4-methylpyridine, 4-isopropenyl-2-methylpyridine, 2-ethyl-4-isopropenylpyridine, 2-acetyl-4-isopropylpyridine, 2,4-diisopropenylpyridine, 2-acetyl-4-isopropenylpyridine, 4-acetyl-2-isopropenylpyridine, 5-[(*Z*)-1-buten-1-yl]-2-propylpyridine, 5-(*E*)-1-buten-1-yl]-2-propylpyridine, 3-[(*Z*)-1-buten-1-yl]-4-propylpyridine, and 3-[(*E*)-1-buten-1-yl]-4-propylpyridine, were identified by comparison of their spectroscopic data with those of synthetic samples. Among them, 2-acetyl-4-isopropenylpyridine is a major component and has a powerful grassy-sweet and minty odor. The typical basic components in spearmint oil were compared with those of peppermint oil. Odor profiles of 15 pyridine derivatives were also described.

The essential oil of *Mentha gentilis* f. *cardiaca* (spearmint MWS) is one of the most important flavoring agents and is used extensively in toothpastes and chewing gums. It is produced mainly in the northwestern and midwestern states of the United States. Although approximately 200 components have been identified from the neutral and acidic parts of spearmint oil (Smith et al., 1963; Nigam and Levi, 1963; Canova, 1971; Tsuneya et al., 1973; Surberg and Kopsel, 1989; Shimizu et al., 1990), there has been only one published study on the basic fraction, from which three pyridine compounds, 3-phenylpyridine, 5-phenyl-2-propylpyridine, and 3-phenyl-4-propylpyridine, were identified in the basic fractions of Midwest Scotch spearmint oil and peppermint oil (Sakurai et al., 1983). The nitrogen compounds seem to greatly contribute to the characteristic odor profile of the spearmint oil because of their powerful and pungent aromas. Therefore, we tried to clarify the detailed profile of the basic fraction of this oil.

This paper concerns the structural elucidation of the basic components in spearmint oil including 11 new pyridine derivatives (1–11) (Figure 1). We also report on the olfactory properties of 15 pyridines and propose the formation mechanisms for 2 of them.

EXPERIMENTAL PROCEDURES

Materials. The oils of *M. gentilis* f. *cardiaca* and *Mentha piperita* (Willamette peppermint) used in this study were cropped in the midwestern United States and in Oregon in 1983, respectively.

Preparation of Basic Fraction. The procedure used to isolate the basic fraction is illustrated in Figure 2. The spearmint oil (49 kg) was shaken with 1 N HCl (7.5 L × 3). After the aqueous layer was washed with toluene (6 L × 3), it was made basic with 2 N NaOH (pH 11) and extracted with distilled ether (8 L × 3). The ether extracts were combined and washed with brine (3 L × 2) and dried over anhydrous $MgSO_4$. After the ether extract was condensed to about 200 mL, the procedure above was repeated to yield 0.5 g of the basic fraction. This oil was separated into 14 fractions, CC-1–14, by column chromatography on silica gel eluted successively with a mixture of hexane and ether, ether, and methanol.

The basic fraction of Willamette peppermint oil (400 g) was prepared in the same way mentioned above.

Apparatus. Qualitative gas chromatography (GC) was performed using a Hitachi K-163 type instrument with flame ionization (FI), flame photometric (FP), and flame thermoionic (FT) detectors and a 40 m × 0.28 mm i.d. glass capillary column coated with SF-96 (0.25-mm film thickness; Chromato Research Inc.). The oven temperature was held at 70 °C for 5 min and programmed from 70 to 220 °C at a rate of 3 °C/min; helium (1 mL/min) was used as a carrier gas. The temperatures of the injector and detector were 250 °C. Quantitative GC was performed using a Hewlett-Packard 5890A instrument equipped with FID and a 60 m × 0.25 mm i.d. fused silica capillary column coated with DB-1 (0.25-mm film thickness; J&W Scientific). The oven temperature was programmed from 50 (5 min isothermal) to 240 °C at a rate of 3 °C/min. Helium (1 mL/min) was used as a carrier gas. The temperatures of the injector and detector were 280 and 300 °C, respectively. Percentages of peak area were calculated with a Hewlett-Packard 3396A integrator. Gas chromatography–mass spectra (GC–MS) were taken on a Hitachi M-80A mass spectrometer combined with a Hitachi 663 gas chromatograph equipped with a 60 m × 0.25 mm i.d. fused silica capillary column coated with DB-1 (0.25-mm film thickness; J&W Scientific). The oven temperature was held at 75 °C for 5 min and programmed to increase 3 °C/min from 75 to 240 °C. The ionization energy was 20 eV. Retention indices were calculated with a Hitachi M-0101 data processor attached to the above GC–MS system on the basis of retention time of *n*-paraffin standards (C_5–C_{25}). For high-resolution mass spectra (HR-MS), the same instrument was used under the condition of ionization energy at 70 eV. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Hitachi R-24B (60 MHz) spectrometer in $CDCl_3$ or CCl_4 with tetramethylsilane as an internal standard. Infrared (IR) spectra were taken on a JASCO IRA-1 spectrometer. Medium-pressure liquid chromatography (MPLC) was carried out using a Merck Lobar column, (Lichroprep Si-60, 40–60 μm, 24 cm × 1 cm i.d.).

Identification of Compounds. The various compounds were identified by the direct comparison of their retention indices (RI) on GC and mass spectra with the authentic data of the synthesized and commercially available samples except for compounds 5 and 6.

Isolation of 2,4-Diisopropenylpyridine (5). The oil of CC-

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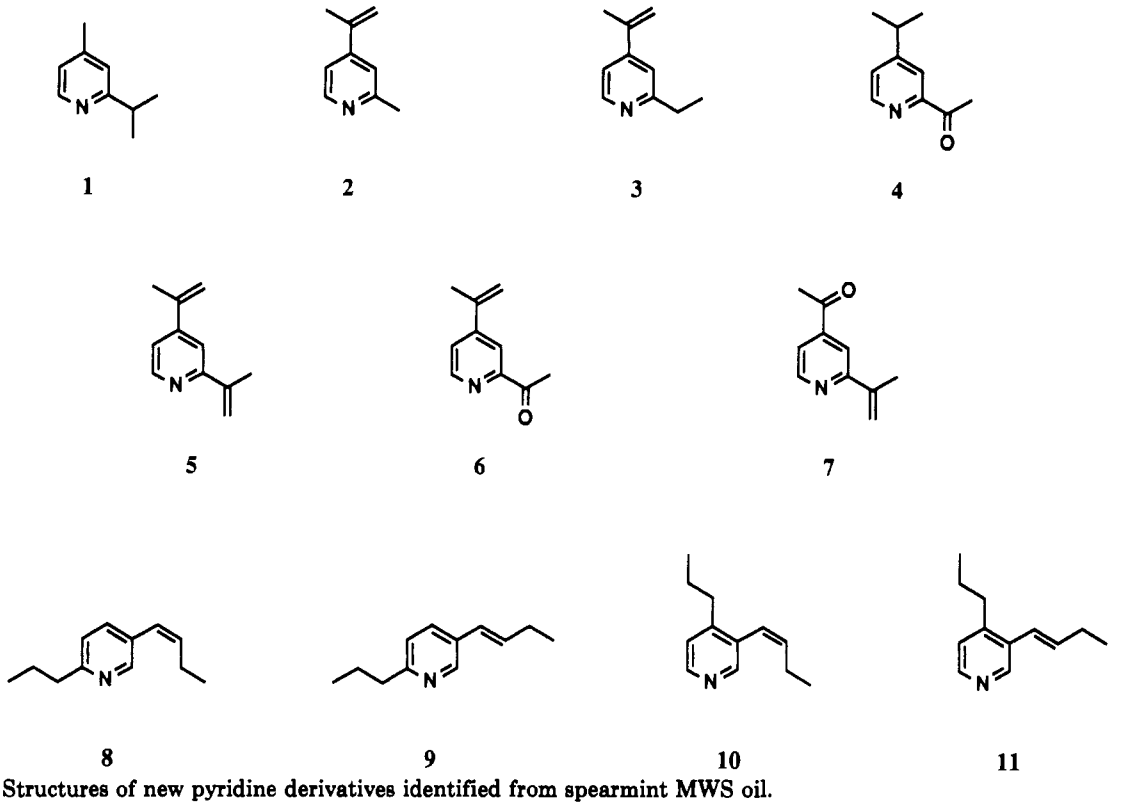


Figure 1. Structures of new pyridine derivatives identified from spearmint MWS oil.

6 and -7 (115 mg) was rechromatographed by MPLC (ether/hexane 8:92) to give 13 mg of compound **5** as a colorless oil: RI = 1293; IR (film) 3100, 1598, 900, 843 cm^{-1}; ^1H NMR (CCl_4) δ 2.07 (m, 3 H, CH_3), 2.17 (m, 3 H, CH_3), 5.16 (m, 2 H, CH_2=), 5.45 (m, 1 H, CH_2=), 5.74 (m, 1 H, CH_2=), 7.04 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.35 (d, J = 1.6 Hz, 1 H, Ar H), 8.36 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 159 (M^+, 100), 158 (86), 144 (18), 119 (25), 91 (13); HR-MS calcd for $C_{11}H_{13}N_1$ $[M]^+$ m/z 159.1047, found $[M]^+$ m/z 159.1004.

Isolation of 2-Acetyl-4-isopropenylpyridine (6). The oil of CC-8 (180 mg) was rechromatographed over silica gel (ether/hexane 1:9) to give 77 mg of compound **6** as a colorless oil: RI = 1323; IR (film) 3100, 3070, 1700, 1598, 1250, 910, 853 cm^{-1}; ^1H NMR (CCl_4) δ 2.17 (br, s, 3 H, CH_3), 2.65 (s, 3 H, CH_3), 5.28 (m, 1 H, CH_2=), 5.61 (m, 1 H, CH_2=), 7.43 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.97 (d, J = 1.6 Hz, 1 H, Ar H), 8.54 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 161 (M^+, 86), 133 (28), 119 (100), 118 (65), 91 (31), 43 (30); HR-MS calcd for $C_{10}H_{11}N_1O_1$ $[M]^+$ m/z 161.0839, found $[M]^+$ m/z 161.0834.

Synthesis of New Pyridine Derivatives. *Preparation of 2-Isopropyl-4-methylpyridine* (**1**). Compound **1** was prepared by regioselective addition of isopropylmagnesium bromide to 1-phenoxycarbonyl salt of 4-picoline according to the modified method of Comins and Abdullah (1982). To a stirred solution of 4-picoline (10 g, 0.11 mol) and CuI (0.8 g, 4 mmol) in 230 mL of THF was added phenyl chloroformate (17 g, 0.11 mol) at -15 °C under Ar. After 5 min, isopropylmagnesium bromide (0.17 mol) in 60 mL of ether was added dropwise over 15 min. The mixture was stirred for 30 min below -5 °C and then at room temperature for another 1 h followed by the addition of aqueous 20% NH_4Cl solution (120 mL). Ether (50 mL) was added, and the organic layer was washed with aqueous 5% NaOH solution, water, and brine. After drying (anhydrous $MgSO_4$), the solution was concentrated to yield 25.5 g of a reddish oil. The crude oil was treated with chloranil (32 g, 0.13 mol) in acetic acid (35 mL) and toluene (100 mL) at 70 °C for 4.5 h. The reaction mixture was cooled and extracted twice with 10% HCl. The acid extracts were washed with toluene and made basic with aqueous 15% NaOH solution and extracted with ether. The ether extracts were washed twice with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the residue was chromatographed over silica gel (hexane/ether 4:1) to give 6.53 g (44%) of **1** as a colorless oil: RI = 1035; IR (film) 3080, 1603, 1562, 820 cm^{-1}; ^1H NMR (CCl_4) δ 1.25 (d, J = 7 Hz, 6 H, two CH_3), 2.25 (s, 3 H, CH_3), 2.94 (dq, J = 7 and 7 Hz, 1 H, CH), 6.76 (d, J = 5 Hz, 1 H, Ar H), 6.81 (s, 1 H, Ar H), 8.26 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 135 (M^+, 40), 134 (42), 120 (100), 107 (28), 93 (23), 77 (3), 65 (5); HR-MS calcd for $C_9H_{13}N_1$ $[M]^+$ m/z 135.1047, found $[M]^+$ m/z 135.1068.

Preparation of 4-Isopropenyl-2-methylpyridine (**2**) *and 2-Ethyl-4-isopropenylpyridine* (**3**). The regioselective alkylations with methyl and ethyl radicals generated from the corresponding carboxylic acid to the 2-position of methyl isonicotinate were performed according to the method of Minisci et al. (1971). To a solution of methyl isonicotinate (5 g, 36 mmol), acetic acid (9 g, 0.15 mol), and $AgNO_3$ (0.9 g, 5 mmol) in 10% H_2SO_4 (100 g), which was heated at 70 °C, was added a solution of $(NH_4)_2S_2O_8$ (12 g, 53 mmol) in H_2O (25 mL) for 15 min at temperatures between 70 and 85 °C. After evolution of CO_2 ceased, the reaction mixture was stirred at 80–87 °C for 30 min and cooled. The solution was washed with ether, and the aqueous layer was made basic with saturated K_2CO_3 solution and extracted three times with ether. The organic layer was washed with brine and dried (anhydrous $MgSO_4$). The solvent was removed under reduced pressure to give 4.2 g of a reddish oil. GC and GC–MS analyses revealed that the oil was a mixture of **12** (41%), methyl 2,6-dimethylisonicotinate (11%), the recovered methyl isonicotinate (29%), and others (19%). Compound **12**: EI-MS m/z (relative intensity) 151 (M^+, 100), 120 (92), 92 (81), 65 (53); HR-MS calcd for $C_8H_9N_1O_2$ $[M]^+$ m/z 151.0633, found $[M]^+$ m/z 151.0673.

The crude product (4 g) was treated with 3 equiv of methylmagnesium bromide in ether (60 mL) at room temperature for 1 h. The reaction mixture was poured into cold diluted HCl. The aqueous layer was made basic with 10% NaOH and extracted three times with ether. The organic layer was washed with brine and dried (anhydrous $MgSO_4$). Evaporation of the solvent gave 2.7 g of a mixture of alcohol derivatives. Compound **13**: IR (KBr) 3200, 2970, 1603, 1175, 1003, 842 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (s, 6 H, two CH_3), 2.47 (s, 3 H, CH_3), 4.55 (br s, 1 H, OH), 7.20 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.29 (d, J = 1.6 Hz, 1 H, Ar H), 8.28 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 151 (M^+, 38), 136 (97), 93 (31), 59 (29), 43 (100); HR-MS calcd for $C_9H_{13}N_1O_1$ $[M]^+$ m/z 151.0997, found $[M]^+$ m/z 151.1003.

The crude product (2.7 g) was refluxed in concentrated H_2SO_4 (13 g) and acetic acid (30 g) for 1.5 h. The reaction mixture was cooled, poured into ice–water, and washed with ether. The

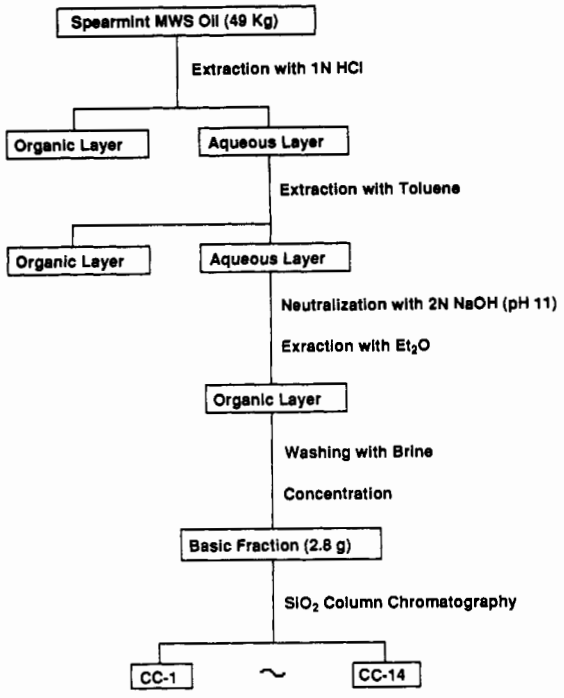


Figure 2. Preparation of basic fraction from spearmint MWS oil.

aqueous layer was made basic with 10% NaOH and extracted three times with ether. The organic layer was washed twice with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the residue was chromatographed over silica gel (ether/hexane 1:3) to give 530 mg (11%) of **2** as a colorless oil: RI = 1100; IR (film) 1600, 1543, 900, 835 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.14 (d, J = 1.6 Hz, 3 H, CH_3), 2.56 (s, 3 H, CH_3), 5.21 (m, 1 H, CH_2=), 5.51 (m, 1 H, CH_2=), 7.12 (d, J = 5 Hz, 1 H, Ar H), 7.16 (s, 1 H, Ar H), 8.43 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 133 (M^+, 100), 132 (30), 118 (19), 117 (18), 91 (33), 65 (11); HR-MS calcd for $C_9H_{11}N_1$ $[M]^+$ m/z 133.0891, found $[M]^+$ m/z 133.0911.

Compound **3** was prepared in 18% overall yield from propionic acid and methyl isonicotinate according to the same reaction procedures mentioned above. Compound **14**; IR (film) 2970, 1735, 1603, 1560, 1290, 1205, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7.5 Hz, 3 H, CH_3), 2.93 (q, J = 7.5 Hz, 2 H, CH_2), 3.93 (s, 3 H, CH_3), 7.62 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.69 (d, J = 1.6 Hz, 1 H, Ar H), 8.66 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 165 (M^+, 90), 164 (100), 137 (53), 106 (22), 77 (20); HR-MS calcd for $C_9H_{11}N_1O_2$ $[M]^+$ m/z 165.0789, found $[M]^+$ m/z 165.0789. Compound **15**: IR (film) 3300, 2970, 1603, 1180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (t, J = 7.5 Hz, 3 H, CH_3), 1.56 (s, 6 H, two CH_3), 2.81 (q, J = 7.5 Hz, 2 H, CH_2), 3.90 (br s, 1 H, OH), 7.17 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.28 (d, J = 1.6 Hz, 1 H, Ar H), 8.21 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 165 (M^+, 100), 164 (81), 150 (36), 108 (21), 43 (42); HR-MS calcd for $C_{10}H_{15}N_1O_1$ $[M]^+$ m/z 165.1153, found $[M]^+$ m/z 165.1159. Compound **3**: RI = 1189; IR (film) 1600, 1543, 900, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (t, J = 7 Hz, 3 H, CH_3), 2.11 (d, J = 1.6 Hz, 3 H, CH_3), 2.83 (q, J = 7 Hz, 2 H, CH_2), 5.18 (m, 1 H, CH_2=), 5.49 (m, 1 H, CH_2=), 7.08 (d, J = 5 Hz, 1 H, Ar H), 7.12 (s, 1 H, Ar H), 8.43 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 147 (M^+, 70), 146 (100), 130 (5), 119 (21), 91 (7); HR-MS calcd for $C_{10}H_{13}N_1$ $[M]^+$ m/z 147.1047, found $[M]^+$ m/z 147.1032.

Preparation of 2-Acetyl-4-isopropylpyridine (**4**). To a solution of 2-acetylpyridine (14.4 g, 0.12 mol), isobutyric acid (32 g, 0.36 mol), and $AgNO_3$ (4 g, 24 mmol) in 10% H_2SO_4 (270 g), which was heated at 70 °C, was added a solution of $(NH_4)_2S_2O_8$ (52 g, 0.23 mmol) in H_2O (120 mL) for 15 min at temperatures between 70 and 80 °C. After evolution of CO_2 ceased, the reaction mixture was stirred at 80 °C for 15 min and cooled. The solution was washed with ether. The aqueous layer was made basic with saturated K_2CO_3 solution and extracted three times with ether. The organic layer was washed with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the residue was chromatographed over silica gel (ether/hexane 3:7) to yield 5.96 g (30.7%) of compound **4** as a colorless oil: RI = 1260; IR (film) 3080, 1700, 1599, 1358, 1202, 843 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (d, J = 7 Hz, 6 H, two CH_3), 2.67 (s, 3 H, CH_3), 2.96 (dq, J = 7 and 7 Hz, 1 H, CH), 7.26 (dd, J = 1.5 and 5 Hz, 1 H, Ar H), 7.87 (d, J = 1.5 Hz, 1 H, Ar H), 8.50 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 163 (M^+, 84), 148 (11), 135 (28), 121 (100), 106 (25), 79 (10), 43 (16); HR-MS calcd for $C_{10}H_{13}N_1O_1$ $[M]^+$ m/z 163.0996, found $[M]^+$ m/z 163.1015.

Preparation of 2,4-Diisopropenylpyridine (**5**), *2-Acetyl-4-isopropenylpyridine* (**6**), *and 4-Acetyl-2-isopropenylpyridine* (**7**). To a solution of dimethyl 2,4-lutidinate (39 g, 0.2 mol) in ether (400 mL) was added dropwise an ethereal solution of 3 M methylmagnesium bromide (400 mL, 1.2 mol) under cooling in an ice–water bath. The reaction mixture was stirred for 1 h at room temperature and then was refluxed for another 1 h. The reaction mixture was poured into cold saturated NH_4Cl. The aqueous layer was extracted three times with a mixed solvent (ether/ethyl acetate 1:2). The combined organic layer was washed with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the brownish residue (39.2 g) was chromatographed over silica gel (ether/hexane 8:2) to give 22.7 g (58%) of compound **16** as colorless crystals and 11.7 g (33%) of a 1:1 mixture of compounds **17** and **18** as a colorless oil. Compound **16**: IR (KBr) 3280, 2960, 1600, 1180, 1102, 955 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 6 H, two CH_3), 1.51 (s, 6 H, two CH_3), 3.60 (br s, 1 H, OH), 5.12 (br s, 1 H, OH), 7.39 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.42 (d, J = 1.6 Hz, 1 H, Ar H), 8.25 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 195 (M^+, 0.8), 180 (100), 165 (12) 138 (20), 137 (22); HR-MS calcd for $C_{11}H_{17}O_2N_1$ $[M]^+$ m/z 195.1257, found $[M]^+$ m/z 195.1226. Compound **17**: EI-MS m/z (relative intensity) 179 (M^+, 100), 164 (58), 137 (72), 78 (35), 59 (43), 43 (63); HR-MS calcd for $C_{10}H_{13}O_2N_1$ $[M]^+$ m/z 179.0945, found $[M]^+$ m/z 179.0978. Compound **18**: EI-MS m/z (relative intensity) 179 (M^+, 5), 164 (100), 121 (33), 59 (13), 43 (5); HR-MS calcd for $C_{10}H_{13}O_2N_1$ $[M]^+$ m/z 179.0945, found $[M]^+$ m/z 179.0913.

The diol **16** (20 g, 0.1 mol) was refluxed in concentrated H_2SO_4 (50 g) and acetic acid (118 g) for 1 h. The reaction mixture was cooled, poured into ice–water, and washed with toluene. The aqueous layer was made basic with 20% NaOH and extracted three times with a mixed solvent (ether/ethyl acetate 1:1). The organic layer was washed with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the residue was chromatographed over silica gel (ether/hexane 1:5) to give 12.5 g (78%) of compound **5** as a colorless oil. The analytical data (RI = 1301, IR, ^1H NMR, and EI-MS) of the synthesized **5** were identical with those of the natural sample.

Dehydration of the mixture of compounds **17** and **18** by the same method mentioned above followed by purification through silica column chromatography (ether/hexane 1:5) afforded compounds **6** and **7** in 37 and 34% yield, respectively. The spectral data (RI = 1323, IR, ^1H NMR, and EI-MS) of the synthesized **6** were identical with those of the natural one. Compound **7** showed the following spectral data: RI = 1333; IR (film) 3100, 1700, 1590, 1550, 1365, 1250, 910, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24 (d, J = 1.6 Hz, 3 H, CH_3), 2.60 (s, 3 H, CH_3), 5.36 (m, 1 H, CH_2=), 5.94 (m, 1 H, CH_2=), 7.56 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.88 (d, J = 1.6 Hz, 1 H, Ar H), 8.74 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 161 (M^+, 100), 160 (70), 146 (12), 118 (30), 117 (20), 91 (18), 43 (25); HR-MS calcd for $C_{10}H_{11}N_1O_1$ $[M]^+$ m/z 161.0839, found $[M]^+$ m/z 161.0867.

To determine the position of the acetyl group on the pyridine ring, compound **6** was also prepared according to the following selective procedure. The regioselective acetylation of methyl isonicotinate with paraldehyde was performed according to the modified method of Giordano et al. (1986). A solution of methyl isonicotinate (41 g, 0.3 mol), trifluoroacetic acid (35 g, 0.3 mol), 70% t-BuOOH (75 g, 0.58 mol), and ferrous sulfate (1.4 g, 5 mmol) was refluxed with paraldehyde (200 g, 1.5 mol) and acetonitrile (700 mL) for 4 h. The solution was concentrated by distillation of the solvent, basified with saturated Na_2CO_3, and extracted twice with toluene. The organic layer was washed with brine and dried (anhydrous $MgSO_4$). The solvent was removed under

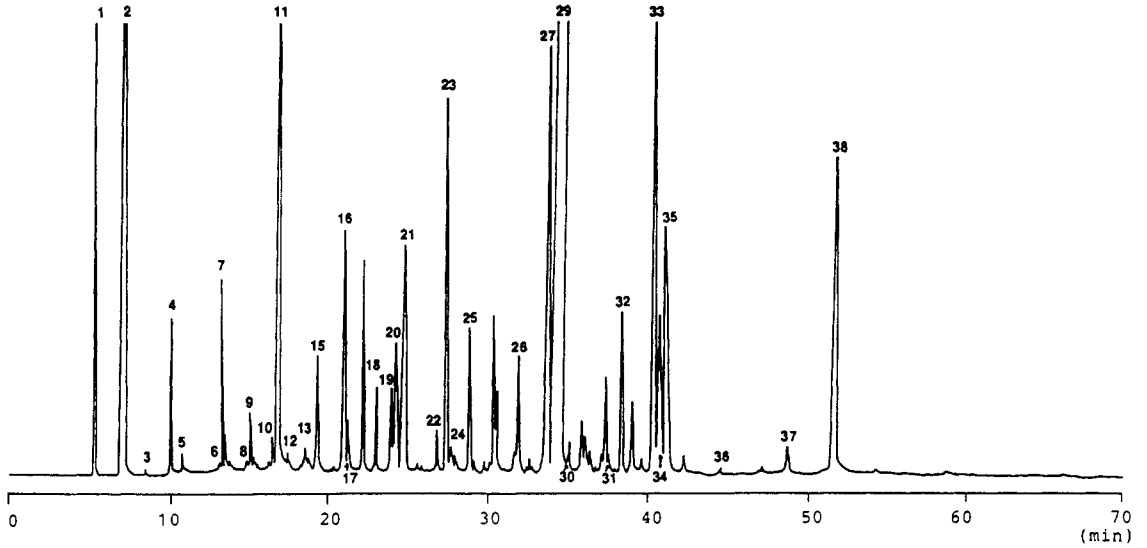
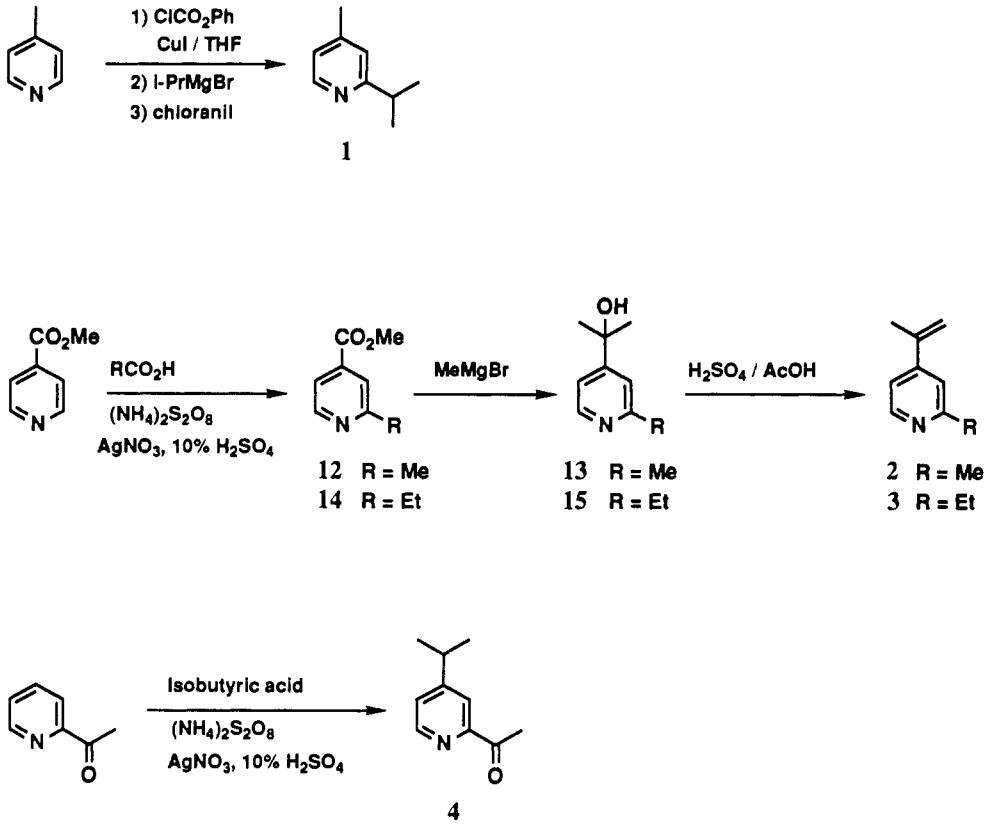


Figure 3. FTD monitored gas chromatogram of basic fraction from spearmint MWS oil on a SF-96 glass capillary column (40 × 0.28 mm i.d.). The numbers correspond to the numbers outlined in Table I.

Scheme I

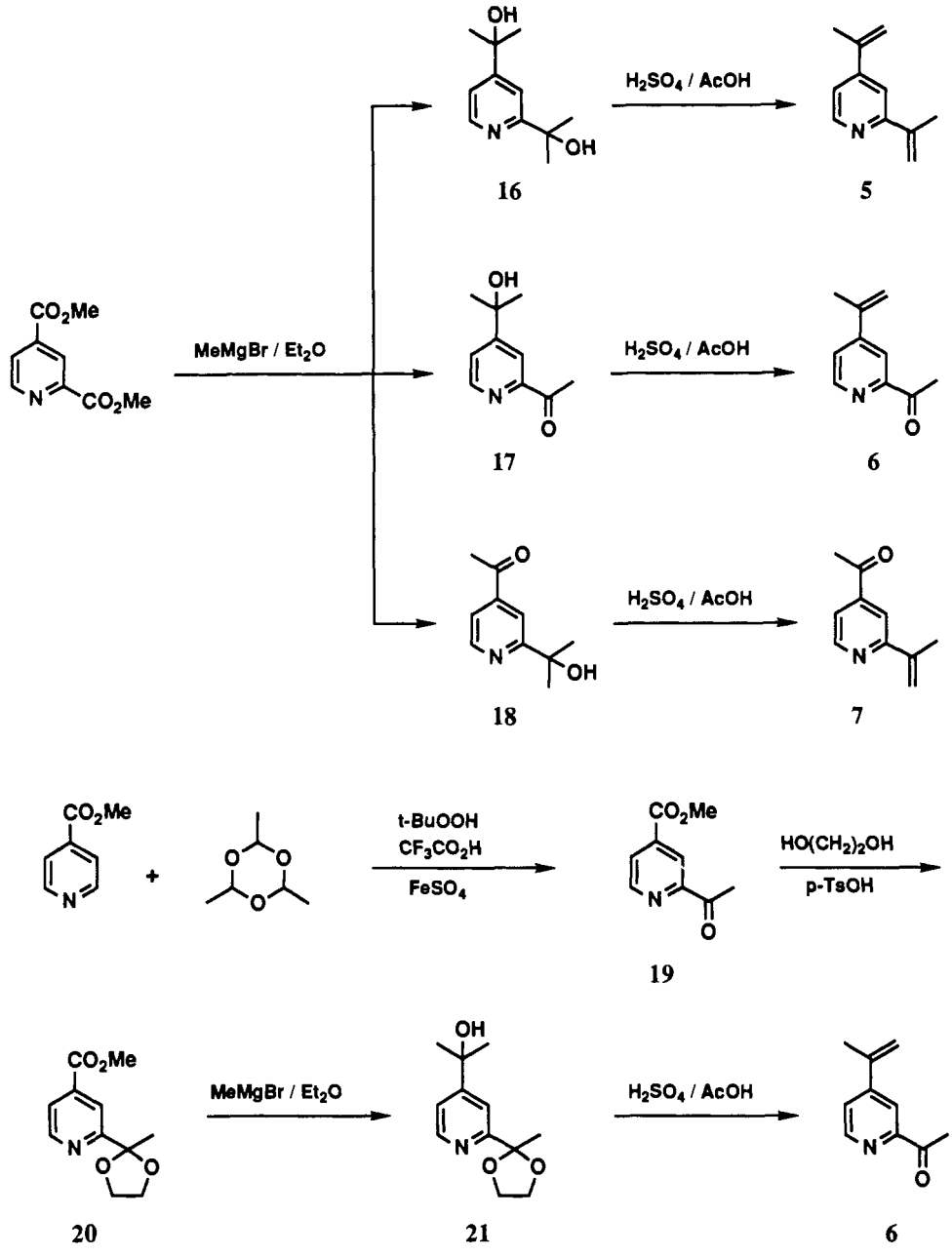


reduced pressure to give 55 g of crude methyl 2-acetylisonicotinate (**19**) as a brownish oil. This oil was submitted to the next procedure without purification. Pure **19** showed the following spectral data: IR (film) 1740, 1703, 1600, 1560, 1315, 1236, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.74 (s, 3 H, CH_3), 4.00 (s, 3 H, CH_3), 8.01 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 8.51 (d, J = 1.6 Hz, 1 H, Ar H), 8.85 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 179 (M^+, 94), 151 (47), 137 (100), 136 (51), 106 (20), 78 (23), 43 (75); HR-MS calcd for $C_9H_9O_3N_1$ $[M]^+$ m/z 179.0582, found $[M]^+$ m/z 179.0623.

A solution of crude **19** (55 g, 0.3 mol), ethylene glycol (65 g, 1.05 mol), *p*-toluenesulfonic acid monohydrate (35 g, 0.18 mol), and benzene (300 mL) was refluxed for 4.5 h, and water formed during the reaction period was azeotropically removed by using a Dean-Stark trap. The reaction mixture was poured into cold water, and the organic layer was extracted three times with 7% H_2SO_4 solution. The aqueous layer was made basic with Na_2CO_3 and extracted with a mixed solvent (toluene/ether 1:1). The combined extracts were washed with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the brownish residue (41 g) was chromatographed on silica gel (hexane/ethyl acetate 1:1) to give 35 g (52%) of **20** as yellowish crystals: IR (KBr) 2990, 1735, 1600, 1305, 1230, 1205, 1035, 875, 700, 615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.73 (s, 3 H, CH_3), 3.97 (s, 3 H, CH_3), 3.84–4.20 (m, 4 H, two CH_2), 7.78 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 8.08 (d, J = 1.6 Hz, 1 H, Ar H), 8.77 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 208 ($M^+ - CH_3$, 7), 180 (12), 136 (9), 87 (100), 43 (47); HR-MS calcd for $C_{10}H_{10}N_1O_4$ $[M - CH_3]^+$ m/z 208.0610, found $[M - CH_3]^+$ m/z 208.0648.

To a solution of compound **20** (35 g, 0.157 mol) in THF (300 mL) was added dropwise an ethereal solution of 3 M methylmagnesium bromide (150 mL, 0.45 mol) at 15–23 °C. The reaction mixture was stirred at 40 °C for 5 h and then was poured into a cold NH_4Cl solution. The organic layer was washed with brine,

Scheme II



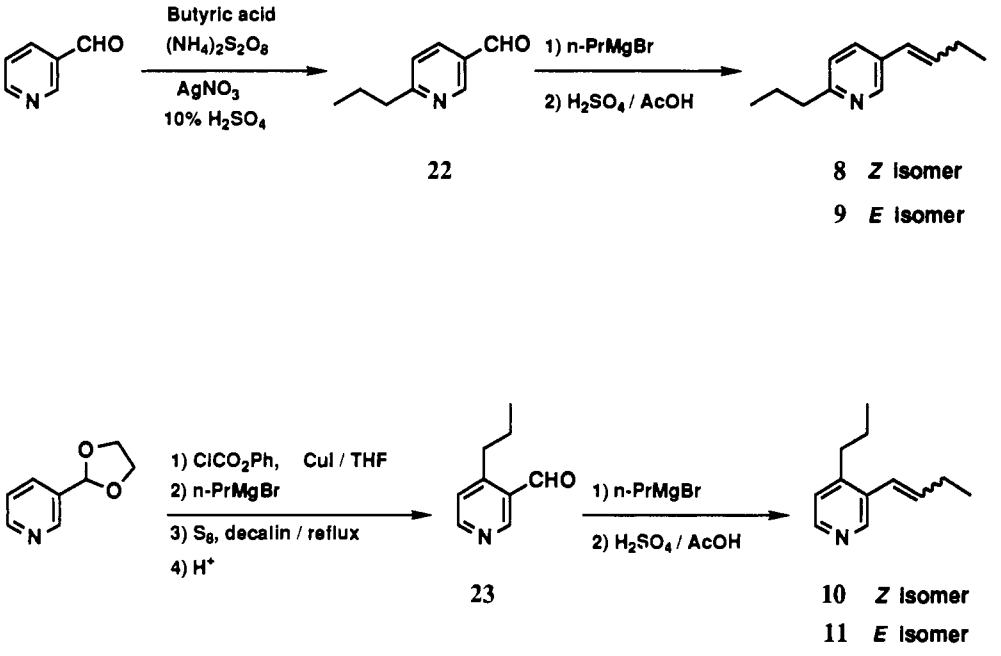
dried (anhydrous $MgSO_4$), and concentrated under reduced pressure to give 30 g of **21** as a reddish oil. This crude oil was submitted to the next reaction procedure without purification. Compound **21**: IR (film) 3360, 2970, 1600, 1180, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56 (s, 6 H, two CH_3), 1.70 (s, 3 H, CH_3), 3.01 (br s, 1 H, OH), 3.77–4.14 (m, 4 H, two CH_2), 7.31 (dd, J = 1.6 and 5 Hz, 1 H, Ar H), 7.63 (d, J = 1.6 Hz, 1 H, Ar H), 8.49 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 208 (M^+ – CH_3, 15), 180 (73), 164 (7), 151 (7), 136 (15), 87 (100), 43 (50); HR-MS calcd for $C_{11}H_{14}N_1O_3$ [M – CH_3]$^+$ m/z 208.0972, found [M – CH_3]$^+$ m/z 208.0931.

A solution of crude compound **21** (30 g, 0.13 mol), concentrated H_2SO_4 (60 g), and acetic acid (140 g) was refluxed for 1.5 h. The reaction mixture was poured into ice–water and extracted with toluene. The aqueous layer was made basic with Na_2CO_3 and extracted three times with a mixed solvent (ether/ethyl acetate 1:1). The combined extracts were washed with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the dark brownish residue (16 g) was chromatographed over silica gel to give 10 g (48%) of **6** as a yellowish oil. The analytical data of compound **6** were identical with those of the natural one.

Preparation of 5-[(Z)-1-Buten-1-yl]-2-propylpyridine (**8**) *and 5-[(E)-1-Buten-1-yl]-2-propylpyridine* (**9**). The alkylation with propyl radical to the 6-position of nicotinaldehyde was performed according to the method mentioned above to afford 6-propylnicotinaldehyde (**22**) as a main product: IR (film) 2970, 2770, 1710, 1595, 1370, 1210 cm^{-1}; ^1H NMR (CCl_4) δ 0.96 (t, J = 7 Hz, 3 H, CH_3), 1.54–2.19 (m, 2 H, CH_2), 2.81 (dd, J = 7 and 8.5 Hz, 2 H, CH_2), 7.17 (d, J = 7.5 Hz, 1 H, Ar H), 7.94 (d, J = 7.5 Hz, 1 H, Ar H), 8.81 (d, J = 2 Hz, 1 H, Ar H), 9.97 (s, 1 H, CHO); EI-MS m/z (relative intensity) 149 (M^+, 1), 148 (6), 134 (25), 121 (100), 92 (5); HR-MS calcd for $C_9H_{10}N_1O_1$ [M – H]$^+$ m/z 148.0762, found [M – H]$^+$ m/z 148.0772.

The subsequent Grignard reaction of compound **22** with propylmagnesium bromide followed by dehydration gave a mixture (7:93) of compounds **8** and **9** as a colorless oil in 5.8% overall yield after purification through silica gel column chromatography (hexane/ether 7:3). The minor isomer **9** showed the following data: RI = 1378; EI-MS m/z (relative intensity) 175 (M^+, 5), 174 (9), 160 (23), 147 (100), 132 (12), 106 (6), 91 (3); HR-MS calcd for $C_{12}H_{17}N_1$ [M]$^+$ m/z 175.1361, found [M]$^+$ m/z 175.1358. The major isomer **9** showed the following data: RI = 1431; IR (film) 1595, 1560, 1485, 960, 910 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, J = 7 Hz, 3 H, CH_3), 1.09 (t, J = 7 Hz, 3 H, CH_3), 1.45–2.50 (m, 4 H, two CH_2), 2.73 (dd, J = 7 and 8 Hz, 2 H, CH_2), 6.29 (m, 2 H, CH=CH), 7.05 (d, J = 7.5 Hz, 1 H, Ar H), 7.58 (dd, J = 1.6 and

Scheme III



7.5 Hz, 1 H, Ar H), 8.45 (d, J = 1.6 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 175 (M^+, 20), 174 (15), 160 (45), 147 (100), 132 (40), 91 (5); HR-MS calcd for $C_{12}H_{17}N_1$ $[M]^+$ m/z 175.1361, found $[M]^+$ m/z 175.1410.

Preparation of 3-[(Z)-1-Buten-1-yl]-4-propylpyridine (10) *and 3-[(E)-1-Buten-yl]-4-propylpyridine* (11). The synthesis of 4-propylnicotinaldehyde (23) was performed according to the modified method of Comins et al. (1984). A solution of nicotinaldehyde ethylene acetal (11 g, 72 mmol), CuI (0.73 g), and dimethyl sulfide (10 mL) in 110 mL of THF was cooled to −15 °C. Phenyl chloroformate (11.4 g, 73 mmol) was added dropwise and the mixture stirred at −15 °C for 10 min. A solution of propylmagnesium bromide (89 mmol) in 40 mL of THF was added dropwise. The mixture was stirred at −10 °C for 15 min followed by the addition of aqueous 20% NH_4Cl solution (180 mL). Ether (150 mL) was added, and the organic layer was washed with 10% HCl and brine. After drying (anhydrous $MgSO_4$), the solution was concentrated to give 18 g of the crude dihydropyridine as a reddish oil. To this oil in decalin (110 mL) was added 2.3 g (72 mmol) of sulfur. The mixture was heated at reflux for 3 h, cooled to room temperature, and extracted four times with 10% HCl. The aqueous layer was made basic, and the organic substances were extracted three times with ethyl acetate. The organic layer was washed with brine and dried (anhydrous $MgSO_4$). Evaporation of the solvent gave 4.5 g of a dark brown oil. To this oil in 70 mL of water was added 7 g of citric acid. The mixture was heated at reflux for 1 h and cooled. The mixture was made basic with Na_2CO_3 and extracted three times with ether. The organic layer was washed with brine and dried (anhydrous $MgSO_4$). After removal of the solvent, the residue was chromatographed over silica gel (ether/hexane 1:4) to give 3.2 g (30%) of 4-propylnicotinaldehyde (23) as a colorless oil: IR (film) 2970, 2770, 1720, 1595, 1215, 840 cm^{-1}; ^1H NMR (CCl_4) δ 1.01 (t, J = 7 Hz, 3 H, CH_3), 1.44–1.98 (m, 2 H, CH_2), 3.00 (dd, J = 7 and 8.5 Hz, 2 H, CH_2), 7.12 (d, J = 5 Hz, 1 H, Ar H), 8.52 (d, J = 5 Hz, 1 H, Ar H), 8.80 (s, 1 H, Ar H), 10.15 (s, 1 H, CHO); EI-MS m/z (relative intensity) 149 (M^+, 90), 148 (95), 134 (100), 130 (55), 120 (25), 106 (30); HR-MS calcd for $C_9H_{11}N_1O_1$ $[M]^+$ m/z 149.0840, found $[M]^+$ m/z 149.0883.

The subsequent Grignard reaction of compound 23 and propylmagnesium bromide followed be dehydration gave a mixture (7:93) of compounds 10 and 11 in 56% yield after purification through silica gel column chromatography. The minor isomer 10 showed the following data: RI = 1383; EI-MS m/z (relative intensity) 175 (M^+, 100), 160 (54), 146 (33), 132 (88), 118 (40), 117 (35); HR-MS calcd for $C_{12}H_{17}N_1$ $[M]^+$ m/z 175.1361, found $[M]^+$ m/z 175.1379. The major isomer 11 showed the following data: RI = 1438; IR (film) 3050, 1595, 1465, 1416, 970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, J = 7 Hz, 3 H, CH_3), 1.07 (t, J = 7 Hz, 3 H, CH_3), 1.60 (m, 2 H, CH_2), 2.17 (m, 2 H, CH_2), 2.56 (dd, J = 7 and 8 Hz, 2 H, CH_2), 6.04 (dt, J = 5.5 and 16 Hz, 1 H, CH=), 6.45 (d, J = 16 Hz, 1 H, CH=), 6.88 (d, J = 5 Hz, 1 H, Ar H), 8.17 (d, J = 5 Hz, 1 H, Ar H), 8.43 (s, 1 H, Ar H); EI-MS m/z (relative intensity) 175 (M^+, 83), 160 (45), 146 (35), 132 (100), 131 (31), 118 (41), 117 (39); HR-MS calcd for $C_{12}H_{17}N_1$ $[M]^+$ m/z 175.1361, found $[M]^+$ m/z 175.1380.

Preparation of Other Reference Compounds. 4-Isopropenylpyridine was prepared in 63% yield by Grignard reaction of methyl isonicotinate followed by dehydration. Spectral data were as follows: RI = 1034; IR (film) 3100, 1595, 1402, 905, 830 cm^{-1}; ^1H NMR (CCl_4) δ 2.08 (m, 3 H, CH_3), 5.14 (m, 1 H, CH_2=), 5.45 (m, 1 H, CH_2=), 7.14 (m, 2 H, Ar H), 8.35 (m, 2 H, Ar H); EI-MS m/z (relative intensity) 119 (M^+, 100), 118 (60), 104 (17), 91 (33), 79 (11).

A mixture (7:93) of Z and E isomers of 3-(1-buten-1-yl)-pyridine was prepared in 63% yield by Grignard reaction of nicotinaldehyde followed by dehydration. Spectral data were as follows. Z isomer: RI = 1131; EI-MS m/z (relative intensity) 133 (M^+, 100), 132 (43), 118 (85), 117 (43), 91 (23), 65 (13). E isomer: RI = 1176; IR (film) 3050, 1570, 1420, 1025, 970, 800, 710 cm^{-1}; ^1H NMR (CCl_4) δ 1.07 (t, J = 7 Hz, 3 H, CH_3), 2.21 (m, 2 H, CH_2), 6.17 (m, 2 H, CH=CH), 6.98 (dd, J = 5 and 8 Hz, 1 H, Ar H), 7.44 (dt, J = 1.6 and 8 Hz, 1 H, Ar H), 8.21 (dd, J = 1.6 and 5 Hz, 1 H, Ar H) 8.35 (d, J = 1.6 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 133 (M^+, 85), 132 (45), 118 (100), 117 (47), 91 (24), 65 (11).

4-Isopropyl-2-methylpyridine was prepared according to the method of Comins and Abdullah (1982) in 52% yield. The spectral data were as follows: RI = 1059; IR (film) 3080, 3030, 2975, 1603, 1560, 920, 830 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20 (d, J = 7 Hz, 6 H, two CH_3), 2.47 (s, 3 H, CH_3), 2.79 (dq, J = 7 and 7 Hz, 1 H, CH), 6.84 (d, J = 5 Hz, 1 H, Ar H), 6.87 (s, 1 H, Ar H), 8.25 (d, J = 5 Hz, 1 H, Ar H); EI-MS m/z (relative intensity) 135 (M^+, 64), 120 (100), 106 (4), 93 (6), 77 (13), 65 (5), 42 (6), 41 (6).

3-Phenyl-4-propylpyridine was prepared from 3-phenylpyridine and butyric acid according to the modified method of Minisci et al. (1971), and it showed the same physicochemical properties as those reported by Sakurai et al. (1983).

5-Phenyl-2-propylpyridine was prepared according to the method of Sakurai et al. (1983), and it showed the same physicochemical properties as those reported.

Other reference standards were obtained from commercial sources.

Odor Profile Evaluation. Odor profiles were evaluated by five trained flavorists. They were asked to describe the odors of

Table I. Basic Components Identified from Spearmint MWS Oil

peak[a]	compound	GC peak area, %	RI_{DB-1} known[b]	RI_{DB-1} unknown[c]	ref
1	pyridine	9.84	702	694	
2	2-methylpyridine	12.45	770	772	
3	3-methylpyridine	tr[d]	819	820	
4	2,6-dimethylpyridine	1.24	846	844	
5	2-ethylpyridine	0.77	863	866	
6	2,5-dimethylpyrazine	0.27	881	873	
7	3-ethylpyridine	2.11	923	921	
8	2-ethyl-6-methylpyrazine	0.32	960	959	
9	2-propylpyridine	0.56	960	961	
10	5-ethyl-2-methylpyridine	0.22	982	986	
11	2-acetylpyridine	1.99	991	993	
12	3-propylpyridine	0.39	1019	1018	
13	4-propylpyridine	0.82	1024	1023	
14	4-isopropenylpyridine	tr	1034	1031	
15	2-isopropyl-4-methylpyridine (1)	0.49	1035	1033	
16	4-isopropyl-2-methylpyridine	1.17	1059	1056	
17	2-butylpyridine	0.24	1065	1067	
18	4-isopropenyl-2-methylpyridine (2)	0.48	1100	1097	
19	3-butylpyridine	0.10	1108	1105	
20	4-butylpyridine	0.72	1124	1123	
21	3-[(*Z*)-1-buten-1-yl]pyridine	0.99	1131	1131	
22	2-pentylpyridine	1.32	1174	1167	
23	3-[(*E*)-1-buten-1-yl]pyridine	1.95	1176	1177	
24	2-ethyl-4-isopropenylpyridine (3)	0.35	1189	1183	
25	quinoline	0.86	1206	1201	
26	2-acetyl-4-isopropylpyridine (4)	0.61	1260	1261	
27	2,4-diisopropenylpyridine (5)	3.46	1301	1293	
28	methyl anthranilate	tr	1311	1312	
29	2-acetyl-4-isopropenylpyridine (6)	33.88	1323	1323	
30	4-acetyl-2-isopropenylpyridine (7)	0.51	1333	1331	
31	5-[(*Z*)-1-buten-1-yl]-2-propylpyridine (8)	0.21	1378	1379	
32	3-[(*Z*)-1-buten-1-yl]-4-propylpyridine (10)	1.33	1383	1388	
33	3-phenylpyridine	5.79	1426	1420	*e*
34	5-[(*E*)-1-buten-1-yl]-2-propylpyridine (9)	0.78	1431	1432	
35	3-[(*E*)-1-buten-1-yl]-4-propylpyridine (11)	2.61	1438	1442	
36	3-benzylpyridine	tr	1500	1497	
37	3-phenyl-4-propylpyridine	0.52	1615	1616	*e*
38	5-phenyl-2-propylpyridine	2.76	1684	1685	*e*

[a] Gas chromatographic peak number in Figure 3. [b] Registered values for authentic compounds. [c] Calculated values for the compounds detected in spearmint MWS oil by the GC–MS system. [d] GC peak area less than 0.01%. [e] Already identified in Sakurai et al. (1983).

the synthesized pyridine compounds by open discussion. Each of the samples was diluted in 5% solution with ethanol.

RESULTS AND DISCUSSION

Characterization of Basic Components. In Figure 2 the analytical steps of the basic fraction of spearmint MWS oil are schematically outlined. The analysis of capillary GC data (SF-96, FID, FPD, and FTD; DB-1; FID) revealed that the calculated amount of the basic fraction was about 10 ppm of spearmint oil. The result of GC (FTD) separation of spearmint basic fraction is illustrated in Figure 3. All 12 fractions (CC-3–14) obtained by silica gel column chromatography were also analyzed by GC and GC–MS. To determine the structures of the components for which authentic MS data were not available, their molecular formulas were clarified by their HR-MS data. The detailed analysis of HR-MS data along with their IR, ^1H NMR, and EI-MS data enabled us to elucidate the structures of the unidentified compounds which were separately synthesized (see Experimental Procedures).

Compound **5** indicated a molecular weight of 159 ($C_{11}H_{13}N_1$, $[M]^+$ 159.1004) in its HR-MS. The IR spectrum showed no absorption of carbonyl group. The ^1H NMR spectrum showed signals at δ 2.07 (m, 3 H, CH_3), 2.17 (m, 3 H, CH_3), 5.16 (m, 2 H, CH_2=), 5.45 (m, 1 H, CH_2=), and 5.74 (m, 1 H, CH_2=), suggesting the existence of two isopropenyl groups. The coupling patterns of three proton signals on pyridine ring were quite similar to those of compound **6**. These spectral data supported the assigned structure of 2,4-diisopropenylpyridine (**5**), which

Table II. Content of the Typical Basic Components in Spearmint and Peppermint Oil

compound	spearmint,[a] ppm	peppermint,[b] ppm
pyridine	0.98	0.26
2-methylpyridine	1.25	0.02
2,5-dimethylpyrazine	0.06	0.02
2-acetylpyridine	0.20	0.07
2-isopropyl-4-methylpyridine (1)	0.05	0.11
4-isopropyl-2-methylpyridine	0.12	1.90
4-isopropenyl-2-methylpyridine (2)	0.05	tr[c]
3-[(*Z*)-1-buten-1-yl]pyridine	0.10	0.15
3-[(*E*)-1-buten-1-yl]pyridine	0.20	0.22
2-ethyl-4-isopropenylpyridine (3)	0.04	tr
quinoline	0.09	0.06
2-acetyl-4-isopropylpyridine (4)	0.06	
2,4-diisopropenylpyridine (5)	0.35	
2-acetyl-4-isopropenylpyridine (6)	3.34	
4-acetyl-2-isopropenylpyridine (7)	0.05	
5-[(*Z*)-1-buten-1-yl]-2-propylpyridine (8)	0.14	0.01
3-[(*Z*)-1-buten-1-yl]-4-propylpyridine (10)	0.02	0.02
3-phenylpyridine	0.58	0.41
5-[(*E*)-1-buten-1-yl]-2-propylpyridine (9)	0.08	0.10
3-[(*E*)-1-buten-1-yl]-4-propylpyridine (11)	0.26	tr
3-phenyl-4-propylpyridine	0.05	0.04
5-phenyl-2-propylpyridine	0.28	0.12
total content[d]	10.00	5.50

[a] Spearmint MWS oil (*M. gentilis* f. *cardiaca*). [b] Willamette peppermint oil (*M. piperita*). [c] Value less than 0.01 ppm. [d] Value including other basic components.

Table III. Odor Profile of Synthetic Pyridine Compounds Identified from Spearmint MWS Oil

compound	odor description
4-isopropenylpyridine	green-bitter, nutty-beany, slightly sweet
4-isopropenyl-2-methylpyridine (2)	ether-like, browny-acidy, radish (ozonelike)
2-ethyl-4-isopropenylpyridine (3)	slightly nutty, herbal, bitter
2,4-diisopropenylpyridine (5)	earthy, sligtly seaweed, somewhat citrus
2-isopropyl-4-methylpyridine (1)	earthy green, somewhat sour and citrus
4-isopropyl-2-methylpyridine	amine-like, ozonous green, violet-perilla
3-[(*Z*) and (*E*)-1-buten-1-yl]pyridine[a]	herbal, white floral like, minty
5-[(*Z*) and (*E*)-1-buten-1-yl]-2-propylpyridine (8 and 9)[b]	somewhat rose, fermented beany, wormwood
3-[(*Z*) and (*E*)-1-buten-1-yl]-4-propylpyridine (10 and 11)[c]	earthy green, green beany, powdery musk like
3-phenylpyridine	nutty, roasted soybean, methyl cinnamate like
3-phenyl-4-propylpyridine	minty, sweet, fermented earthy
5-phenyl-2-propylpyridine	green tomato leaf, slightly methyl cinnamate like
2-acetyl-4-isopropenylpyridine (6)	grassy-sweet, minty, somewhat amber-like
4-acetyl-2-isopropenylpyridine (7)	weak herbal green, fermented roast
2-acetyl-4-isopropylpyridine (4)	grassy-green leaf, green herbal, somewhat violet

[a] A mixture (*Z*/*E* = 7:93). [b] A mixture (*Z*/*E* = 7:93). [c] A mixture (*Z*/*E* = 7:93).

was confirmed by the synthesis described under Experimental Procedures. The spectral data of the synthesized **5** were absolutely identical with those of the natural one.

The major compound **6** indicated a molecular weight of 161 ($C_{10}H_{11}N_1O_1$, $[M]^+$ 161.0834) in its HR-MS. The IR spectrum showed strong absorptions of acetyl carbonyl group at 1700 and 1250 cm^{-1}. The ^1H NMR signals at δ 2.17 (br s, 3 H), 5.28 (m, 1 H), and 5.61 (m, 1 H) revealed the existence of an isopropenyl group. The signals at δ 7.43 (dd, J = 1.6 and 5 Hz, 1 H), 7.97 (d, J = 1.6 Hz, 1 H), and 8.54 (d, J = 5 Hz, 1 H) suggested that the acetyl and the isopropenyl groups are attached at the 2- and 4-positions or the reverse on the pyridine ring, respectively. These spectral data supported the structural assignments of 2-acetyl-4-isopropenylpyridine (**6**) and 4-acetyl-2-isopropenylpyridine (**7**). The structure of compound **6** was confirmed by the synthesis as shown in Scheme II. The spectral properties of the synthetic product were absolutely identical with those of the isolated one from spearmint oil.

Thirty-eight constituents, their contents (GC areas), and RI data are given in Table I. Pyridine and its derivatives not only occupy a major portion of the components of spearmint basic fraction but also are responsible for the characteristic odor of spearmint basic fraction. Except pyridine derivatives, only four kinds of other compounds were found among 38 compounds, and their total content was only 1.45%. A total of 35 compounds was identified for the first time from spearmint oil, and 11 pyridine derivatives (1–11) were new compounds which have not been reported so far. It is particularly noteworthy that 2-acetyl-4-isopropenylpyridine (**6**) accounts for a third of the basic components.

Pyridine and five other derivatives, 2,6-dimethylpyridine, 5-ethyl-2-methylpyridine, 3-phenylpyridine, 3-phenyl-4-propylpyridine, and 5-phenyl-2-propylpyridine, had been already identified as the basic components of Mitcham peppermint oil (Takahashi et al., 1980; Sakurai et al., 1983). It is also reported that phenylpyridine derivatives have very strong characteristic odors and are representative of an odor profile of the basic fraction of peppermint oil.

While 4-isopropyl-2-methylpyridine (Comins and Abdullah, 1982) and (*Z*)- and (*E*)-3-(1-buten-1-yl)pyridine (Steiner et al., 1963) had been already synthesized, to our knowledge this is the first report of the identification of these compounds from nature. Pyridine and its 2-methyl-, 3-methyl-, 2-ethyl-, 2-acetyl-, 2,6-dimethyl-, and 5-ethyl-2-methyl derivatives have been reported as the volatile components of various kinds of cooked or roasted foods [i.e., roasted cocoa (Vitzthum et al., 1975a)], tea (Vitzthum et al., 1975b), and whiskey (Nishimura and Masuda, 1971). The occurrences of these pyridine derivatives including the other known ones in Table I were well reviewed by Maga (1981) and Vernin (1982). Their olfactory properties were also reported by Pittet and Hruza (1974) and Winter et al. (1972, 1975, 1976a,b).

The contents of 22 typical basic components were quantitatively determined both in spearmint oil and in peppermint oil. The results are listed in Table II. There are both qualitative and quantitative differences between the two oils. Compounds **4**–**7** were not found in Willamette peppermint oil. The total amount of basic components in spearmint oil was nearly twice as much as that found in peppermint oil. This remarkable difference is mainly caused by the four characteristic components of spearmint oil listed above. These compounds possess two characteristic functional groups (acetyl, isopropenyl, and isopropyl) at the 2- and 4-positions on the pyridine ring, respectively. Considering that *l*-carvone, which is a major component of spearmint oil (60–70% of the oil), is postulated to be the precursor of these compounds, it is interesting that compounds **2** and **3** were also identified in peppermint as well as spearmint oil.

Odor Evaluation. Odor profiles of the 12 newly identified pyridine derivatives together with 3-phenylpyridine, 3-phenyl-4-propylpyridine, and 5-phenyl-2-propylpyridine are listed in Table III. The odor properties of the listed pyridine derivatives vary markedly. Winter et al. (1972, 1975, 1976a,b) have reported that 2-acetylpyridine has a strong roasted and coffee-like odor, while that of 2-acetyl-6-methylpyridine was chocolate-like. In contrast, the 4-alkyl-2-acetylpyridines **4** and **6** have powerful grassy-sweet or green and minty aromas. In particular,

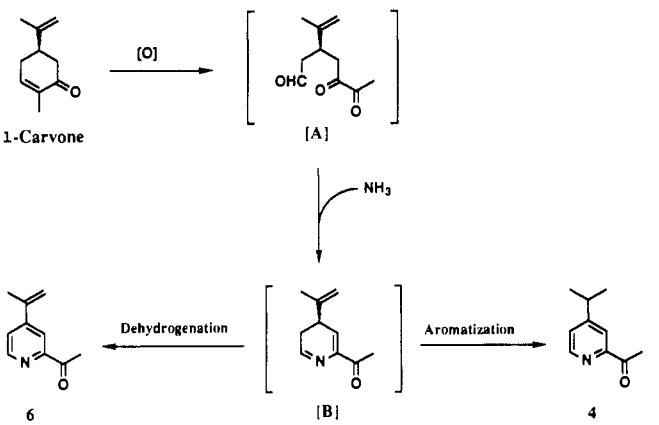


Figure 4. Plausible formation mechanism of 2,4-disubstituted pyridines **4** and **6**.

grassy-sweet or green and minty aromas. In particular, compound **6** seems to contribute greatly to the characteristic odor profile of the basic fraction of spearmint oil because of its odor property and its amount.

Possible Biosynthetic Pathway. It is well-known that pyridines in foods and essential oils are mainly formed from carbonyl compounds and ammonia (Vernin, 1982). As can be seen from Table I, a feature of the basic components of spearmint oil is the presence of the 2,4-disubstituted pyridine derivatives, which are rare in nature. These compounds were not found in peppermint oil. Therefore, the formation mechanism of their characteristic skeleton can be plausibly explained as shown in Figure 4. That is, the oxidative degradation product of *l*-carvone reacts with ammonia to give dihydropyridine intermediate B. Aromatization of B affords 2-acetyl-4-isopropylpyridine (4). On the other hand, dehydrogenation of B produces 2-acetyl-4-isopropenylpyridine (6). Other 2-alkyl-4-isopropenylpyridines **2**, **3**, and **5** are probably formed by another mechanism from *l*-carvone.

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Received for review February 25, 1992. Accepted June 25, 1992.

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