Note

Optical Isomers of Methyl Jasmonate in Tea Aroma

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Received July 27, 1995

Two epimers of methyl jasmonate were optically resolved by capillary gas chromatography, using heptakis (2,3,6-tri-O-methyl)-\(\beta\)-cyclodextrin as the chiral stationary phase. In the tea volatile concentrates, both of these epimers were present as only one enantiomer, their absolute configurations being ascertained as (\(-\))-(1R,2R)-methyl jasmonate and (\(+\))-(1R,2S)-methyl epijasmonate.

The thermal isomerization of methyl epijasmonate to methyl jasmonate was also clarified by optically resolved gas chromatography to have occurred at the asymmetric carbon of the C-2 position that is connected to the carbonyl group.

Key words: methyl jasmonate; methyl epijasmonate; chiral separation; thermal isomerization; tea aroma formation

The two epimers of methyl jasmonate (methyl 3-oxo-2(2(Z)-pentenyl)cyclopentane-1-acetate, MJs) shown in Fig. 1 have been identified as important flavor constituents of semi-fermented tea due to their characteristic jasmine-like aroma.\(^3\) They have also been detected in the pheromone gland of a male oriental fruit moth [Grapholita molesta (Busck)] and in lemon peel.\(^2\)\(^,\)\(^3\) Acree et al. have pointed out that methyl epijasmonate (epi-MJ) had a 400-times stronger odor than methyl jasmonate (MJ), and that the enantiomer of (\(+\))-(1R,2S)-epi-MJ had the strongest odor and pheromone activity.\(^4\) Based on this, Kobayashi et al. have claimed epi-MJ to be more important than MJ for the semi-fermented tea aroma,\(^5\) although their absolute configurations have not been clarified. It is also known that epi-MJ is thermodynamically unstable and is easily epimerized to MJ by heating during preparation and analysis. This epimerization is thought to occur at the C-2 position.\(^6\)

There have been several reports on the optical resolution of MJs by high-resolution liquid chromatography (HPLC) after converting to the diastereomeric ketal of (\(-\))-2,3-butanediol or to the diastereomeric esters of (\(-\))-borneol.\(^7\)\(^,\)\(^8\) Furthermore, their direct enantiomeric separation by HPLC in a Chiralpak AS column\(^9\) or by capillary gas chromatography, using octakis (2-O-methyl-3,6-di-O-pentyl)-\(\beta\)-cyclodextrin as the chiral stationary phase,\(^10\) has been reported. In the present study, we attempted the direct stereodifferentiation of the four optical isomers of MJs in oolong tea volatile concentrates, using heptakis (2,3,6-tri-O-methyl)-\(\beta\)-cyclodextrin as the chiral capillary gas chromatographic stationary phase (Chrompack, B-CD column), and tried to clarify the absolute configurations of MJs as tea aroma constituents and the pathway for thermal isomerization of epi-MJ to MJ.

The tea samples used in this study were two varieties of commercial oolong tea (Huang Jin Gui and Tie Guan Yin) imported from China in 1993. We prepared the volatile concentrates from each tea sample by the method of steam distillation under reduced pressure (SDRP) that was described in the previous paper.\(^11\)

Synthetic standards of (\(+\)), (\(-\))-MJ, and (\(+\)), (\(-\))-epi-MJ were presented by Hasegawa Perfumery Co. A single and a mixture of the four stereomers in a suitable proportion were each subjected to chiral GC. Figure 2 shows the chiral gas chromatogram of the mixture. Both MJ and epi-MJ were separated into their enantiomers in the B-CD column, while the elution order was verified as (\(-\))-(1R,2R)-MJ, (\(+\))-(1S,2S)-MJ, (\(+\))-(1R,2S)-epi-MJ, and (\(-\))-(1S,2R)-epi-MJ by a direct comparison with the Kovats indices of the standards, as summarized in Table I.

We performed mass chromatography on the tea volatile concentrates to identify the GC peaks of MJ and epi-MJ with \(m/z\) 83, 95, 151, and 224, since there were several peaks appearing near the retention times of the MJ and epi-MJ standards. As shown in Fig. 3, both MJ and epi-MJ in the tea volatile concentrates were observed as a single peak by chiral GC. According to co-chromatography with the synthetic optical isomers, their absolute

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**Fig. 1.** Structures of Methyl Jasmonate and Methyl Epijasmonate.

**Fig. 2.** Chiral Chromatogram of a Mixture of the Four Stereomers of MJs.

**Table I.** Kovats Indices (K1) of the Four Stereomers of MJs in a B-CD Column

<table>
<thead>
<tr>
<th>Configuration</th>
<th>K1</th>
</tr>
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<tbody>
<tr>
<td>((-))-(1R,2R)-MJ</td>
<td>1849</td>
</tr>
<tr>
<td>((+))-(1S,2S)-MJ</td>
<td>1860</td>
</tr>
<tr>
<td>((+))-(1R,2S)-epi-MJ</td>
<td>1889</td>
</tr>
<tr>
<td>((-))-(1S,2R)-epi-MJ</td>
<td>1891</td>
</tr>
</tbody>
</table>
was the sole form contributing to the oolong tea aroma, which agrees with the result of Kobayashi et al.\(^2\) already described.

Since \textit{epi-MJ} is easily epimerized to MJ by heating, large amounts of MJ in the tea aroma are presumed to be isomerized during the tea manufacturing process. As shown in Fig. 1, there is a carbonyl group connected to the asymmetric carbon of the C-2 position, so that thermal isomerization occurs only at the C-2 position via the enol form, while the absolute configuration of the C-1 position would remain unchanged. The presence of only the (1R)-form of both MJ and \textit{epi-MJ} in the tea aroma also supports this conjecture. In order to clarify it, we heated standard (\(+\)-(1R,2S)-\textit{epi-MJ}, including a little of its enantiomer and its C-2 epimer, at 95°C in a sealed glass tube. Figure 4 shows chiral gas chromatograms of unheated and heated \textit{epi-MJ}. Both of the two enantiomers of \textit{epi-MJ} decreased, while those of MJ increased. The conversion ratios of (\(+\)-(1R,2S)-\textit{epi-MJ} to (\(-\)-(1R,2R)-MJ and of (\(+\)-(1S,2R)-\textit{epi-MJ} to (\(+\)-(1S,2S)-MJ were 85.1% and 88.1%, respectively, conclusively supporting the foregoing conjecture.

\textit{Epi-MJ} and other \textit{cis}-2,3-substituted cyclopentanone derivatives are known to occur widely in plants, some of which possess growth-regulating properties.\(^1\)\(^-\)\(^3\)\(^4\) The biosynthetic process from linolenic acid by a series of enzymatic reactions has been proposed to involve a \textit{cis}-configuration.\(^4\)\(^\text{a}\) These results suggest (\(+\)-(1R,2S)-\textit{epi-MJ} in a tea volatile concentrate to be an original product biosynthesized from linolenic acid, and that (\(-\)-(1R,2R)-MJ would be transformed by heating during the tea-manufacturing process. Since the odor of (\(+\)-(1R,2R)-MJ is significantly weaker than that of (\(+\)-(1R,2S)-\textit{epi-MJ}, close temperature control of the heating process is considered indispensable to produce high-quality oolong tea.

**Experimental**

Gas chromatography (GC). Based on the report by Kobayashi et al.,\(^1\) the injection temperature was set at 170°C to avoid the epimerization of \textit{epi-MJ} during the gas chromatographic analysis.

a) Conventional analysis. A Shimadzu GC-7A gas chromatograph equipped with FID was used with the following GC conditions: column, 50 m x 0.25 mm (i.d.) fused silica capillary type coated with PEG-20M (CP-WAX); N\(_2\) carrier gas flow rate, 1.1 ml min; split ratio, 35:1; column temperature, held at 60°C for 4 min and raised to 180°C at a rate of 2°C min.

b) Chiral analysis. A Shimadzu GC-9A gas chromatograph equipped with a CP-Cyclohexane-B-236 M-19 capillary column of 50 m x 0.25 mm (i.d.) (Chrompack, B-CD column) and FID was used. The oven temperature was held at 145°C, the N\(_2\) carrier gas flow rate was 1.05 ml min. and the split ratio was 29:1.

Gas chromatography-mass spectrometry (GC-MS). A Hewlett-Packard 5972 mass spectrometer interfaced to a Hewlett-Packard 5990 gas chromatograph was used for MS identification. The GC conditions were the same as those for the corresponding GC analyses, except that He was used as the carrier gas.

**Acknowledgment.** We thank Dr. Yajima of Hasegawa Perfumery Co. for providing the four methyl jasmonate stereomers as standards.

**References**

692 (1962).