Short Communication

Geranyl 6-O-α-L-Arabinopyranosyl-β-D-glucopyranoside Isolated as an Aroma Precursor from Leaves of a Green Tea Cultivar

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Received October 23, 1995

A new glycosidic aroma precursor was isolated from green tea leaves (Camellia sinensis var. sinensis cv. Yabukita) along with the known primeverosides of cis-linalool 3,6-oxide, linalool and geraniol. These glycosides were separated by chromatographic isolation on Amberlite XAD-2, ODS flash chromatography, and finally HPLC. The chemical structure of the new unknown glycoside was confirmed as geranyl 6-O-α-L-arabinopyranosyl-β-D-glucopyranoside (geranyl β-vicianoside) by spectrometric analyses and by an enzymatic hydrolysis with glycosidase followed by GC-MS and HPLC analyses. Moreover the vicianoside was hydrolyzed with acetone powder obtained from fresh tea leaves to generate the same compounds, suggesting this glycoside to be a tea aroma precursor.

Key words: green tea; Camellia sinensis; geranyl 6-O-α-L-arabinopyranosyl-β-D-glucopyranoside; geranyl β-vicianoside; monoterpane alcohol glycoside

In recent years, some of the mechanism for tea aroma formation was proved to be an enzymatic hydrolysis of glycosides that are known as aroma precursors. As those glycosides, we have isolated β-D-glucopyranosides of benzyl alcohol and (Z)-3-hexenol from fresh tea leaves (cv. Yabukita), and Sakata et al. have reported the isolations of β-primeverosides (6-O-β-D-glucopyranosyl-β-D-glucopyranoside) of geraniol, linalool, 2-phenylethanol, benzyl alcohol, and trans- and cis-linalool 3,6-oxide from oolong tea leaves (Camellia sinensis var. sinensis cv. Shuxian and cv. Maxie). Disaccharides of tea aroma compounds have been isolated from oolong tea leaves, but not from green tea leaves. It has been reported that considerable amounts of geraniol, linalool, and linalool oxides are also freed by the glycosidase treatment of the glycosidic fractions in the green tea leaves. Particularly the amounts of geraniol were the same, as much as that of benzyl alcohol after the enzymatic hydrolysis. However, as geranyl β-D-glucopyranoside has not been identified, geraniol was considered to exist in green tea leaves as another glycoside. In this paper we report the isolation of a new geranyl glycoside as aroma precursor together with the several primeverosides that have been found in oolong tea leaves but not in green tea leaves. The structures of the isolated glycosides were confirmed by NMR and by an enzymatic hydrolysis (Rohapect D5L) followed by GC-MS analysis for volatile aglycones and by HPLC for sugar components.

Fresh tea leaves were immediately steamed as usual in the green tea manufacturing process to deactivate enzymes. The dry leaves (2.36 kg, Camellia sinensis var. sinensis Yabukita; harvested in May, 1993, in Shizuoka, National Research Institute of Tea) were crushed with a Willey-style crusher and extracted three times with boiling water (18, 12, 9 liters) for 10 min. The hot water extract was added to MeOH to make an 80% MeOH solution to deposit protein. The solution was concentrated and extracted with chloroform by a continuously stirred extraction (24 h) to remove caffeine. The aqueous phase was then adjusted to pH 7.0 with diluted NaHCO3 and saturated basic lead acetate was added to the solution to deposit catechins and pigments. After centrifugation (1500 × g, 10 min), the supernatant was chromatographed on an Amberlite XAD-2 column (H2O, pentane, and MeOH).

The crude glycosidic fraction eluted with MeOH was concentrated (2.84 g) and a part of the concentrate (993 mg) analyzed by ODS flash chromatography (ODS-SS-1020; Senshu Scientific Co., Ltd.) with stepwise elutions of MeOH-H2O (20, 30, 40, 50, 100%). Main fractions, 30%, 40%, and 50% MeOH eluates, were analyzed by HPLC on an ODS column (Pegasil ODS, 20 μ × 250 mm; gradient elution of 20–40%, 30–100%, and 30–100% CH3CN-H2O) using a UV detector (210 nm) to give compound 1 (12.7 mg) from 30% MeOH eluate, compounds 2 (9.4 mg) and 4 (3.2 mg) from 40% MeOH elute and compound 3 (6.1 mg) from 50% MeOH elute.

HRFABMS data were taken on a JEOL JMS-AX505W A.

Table 1. HRFABMS Data for Compound 1–4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>m/z</th>
<th>.△m/mu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C21H36O11</td>
<td>465.2368 [M+H]+</td>
<td>+3.3</td>
</tr>
<tr>
<td>2</td>
<td>C21H36O10Na</td>
<td>471.2161 [M+Na]+</td>
<td>-4.5</td>
</tr>
<tr>
<td>3</td>
<td>C21H36O10Na</td>
<td>471.2256 [M+Na]+</td>
<td>+4.8</td>
</tr>
<tr>
<td>4</td>
<td>C21H36O10Na</td>
<td>471.2248 [M+Na]+</td>
<td>+1.8</td>
</tr>
</tbody>
</table>

Later we found that the molecular formula of compound 1 was confirmed to be C21H36O11 from HRFABMS data (Table 1) and its
structure was identified as cis-linalool 3,6-oxide 6-O-β-D-xylopyranosyl-β-D-glucopyranoside by comparing $^1$H-NMR and $^{13}$C-NMR spectra with those previously reported.\(^8\)

The molecular formula of compound 2 was confirmed to be C$_{21}$H$_{36}$O$_{10}$ from HRFABMS data (Table I) and its structure was identified as linalyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside by comparing $^1$H-NMR and $^{13}$C-NMR spectra with those previously reported.\(^8\)

The molecular formula of compound 3 was confirmed to be C$_{21}$H$_{36}$O$_{10}$ from HRFABMS data (Table I) and its structure was identified as geranyl 6-O-β-D-xylopyranosyl-β-D-glucopyranoside by comparing $^1$H-NMR and $^{13}$C-NMR spectra with those previously reported.\(^8\)

The compounds 1, 2, and 3 had been isolated from oolong tea leaves, the primeroviosides of monoterpene alcohols were also shown to be present in green tea leaves as well as in oolong tea leaves.

Compound 4 was obtained in a pure state by repeating HPLC. Its molecular formula was confirmed to be C$_{21}$H$_{36}$O$_{10}$ from HRFABMS data (Table I) and $^1$H-NMR and $^{13}$C-NMR spectra (Table II) suggested to have a geranyl structure and two anomic protons (δ 4.28d and 4.35d) and carbons (δ 101.3 and 104.4). These signals showed that compound 4 was a geranyl glycoside of a disaccharide. In the $^1$H-NMR spectrum, the coupling constant (8.0 Hz) of the anomic proton signal at 4.35 ppm established the β-configuration of the glycosidic linkages. The signals of aglycone and glucoside moiety of compound 4 were similar to those of compound 3, however, the signals of xylopyranoside structure of compound 3 were significantly different from those of compound 4, although the HRFABMS data suggest that compound 4 has a pentose along with a glucose.

We tried enzymatic hydrolysis with glycosidase (Rohapopt D5L fungus, Roehm, Germany)\(^9\) on this pure sample to confirm its chemical structure. The liberated aglycone was analyzed by GC-MS [GC part: Hewlett Packard 5890; PEG-20M (DB-WAX) fused silica capillary column, 60 m × 0.25 mm i.d.; He flow rate, 1.0 ml/min.; splitless; 60°C (4 min hold) → 180°C (2°C/min); Injection, 200°C-column/MS part: Hewlett Packard 5972 mass spectrometer and a Hewlett Packard 5972 data processing system], and only one peak of geraniol was identified on GC. The liberated monosaccharides were analyzed by Sugar Analysis HPLC [Hitachi HPLC L-6300; Hitachi Gel M3013-N, 4.6 × 150 mm, 70°C; Flow rate, 0.4 ml/min. (Eluent: sodium borate buffer), Reagent: phenylhydrazine reagent with flow rate of 0.5 ml/min); Detector, UV 365 nm, FL Ex 330 nm, Em 405 nm], and were confirmed to be a 1:1 mixture of glucose and arabinose. Moreover, we attempted an enzymatic hydrolysis of compound 4 using the acetone powder prepared from fresh green tea leaves as a natural crude enzyme system.\(^9\) The hydrolyzed products were completely the same as those obtained as above. This result shows that compound 4 is also a precursor of tea aroma.

Then we attempted COSY, HMQC, and HMBC spectra analyses to allow the full assignments of all the $^1$H and $^{13}$C signals. The correlation between carbon and proton signals of compound 4 was done with the HMBC spectrum. In the HMBC spectrum of compound 4, the anomeric proton H-1" at δ 4.28 ppm has cross peaks with two methylene carbons of C-6' at δ 69.2 ppm and C-5" at δ 67.0 ppm. Another anomic proton H-1' at δ 4.35 ppm showed a cross peak with C-1 of aglycone at δ 66.5 ppm. Thus the gross structure of compound 4 was deduced as shown in Table II. From these assignment, a pentose of compound 4 was confirmed to be an arabinopyranose. The α-configuration of the arabinopyranose moiety was deduced from the coupling constant (7.3 Hz) of the anomeric proton signals at 4.28 ppm. Finally the structure of compound 4 was identified geranyl 6-O-β-D-arabinopyranosyl-β-D-glucopyranoside (geranyl β-vicianoside) shown in the Fig. β-Vicianosides have also been reported to exist in flowers of Gardenia jasminoides\(^11\) and fruits of Rubus idaeus\(^12\) as a flavor precursor. The spectra of NMR coincided well with the data of those previously reported.

As mentioned above, geraniol is present as an aglycone of primeveroside in both oolong and green tea leaves and this is the first identification of β-vicianoside in fresh tea leaves. Geraniol is an important aroma constituent to every kind of tea. The presence of geranyl glycosides of various disaccharides in tea leaves seems interesting for studying the aroma formation mechanism and the difference of aroma patterns depending on the cultivars.

References

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A New Glycoside from Tea Leaves


