**Note**

(S)-Linalyl, 2-Phenylethyl, and Benzyl Disaccharide Glycosides Isolated as Aroma Precursors from Oolong Tea Leaves

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### Abstract

Three glycosides, 6-O-β-d-xylpyranosyl-β-d-glucopyranosides (β-primeverosides) of the aroma constituents, linalool, 2-phenylethanol, and benzyl alcohol, were isolated as aroma precursors from the tea leaves (*Camellia sinensis* var. *sinensis* cv. Shuixian and Maoxie, cultivars for oolong tea). The isolation was guided by acid or enzymatic hydrolysis, and subsequent GC and GC-MS analyses. The linalyl glycoside is the first example of naturally occurring (S)-linalyl β-primeveroside.

The hydrolysis of glycosides by endogenous enzymes has been proposed to be the most plausible mechanism for the formation of an alcoholic tea aroma. While glycosides of benzyl alcohol and (Z)-3-hexenol have been isolated from green tea leaves (cv. Yabukita), we have recently isolated a disaccharide glycoside, geranyl 6-O-β-d-xylpyranosyl-β-d-glucopyranoside, as an aroma precursor from oolong tea leaves (cv. Shuixian). We now report the isolation of 3 other kinds of important aroma precursors of linalool, 2-phenylethanol, and benzyl alcohol from oolong tea leaves.

Oolong tea has a floral aroma mainly composed of monoterpene alcohols and aromatic alcohols. After plunging, tea leaves (*C. sinensis* var. *sinensis* cv. Shuixian) were heated by pan-firing to inactivate the enzymes immediately and then dried in hot air. Two kg (dry weight) of the sample was extracted with hot water.

The isolation procedure for a linalyl glycoside was similar to that for the geranyl glycoside: the extract was subjected to column chromatography with active carbon, Amberlite XAD-2, Sephadex LH-20, and ODS. The aroma precursors of monoterpene alcohols were detected by acidic hydrolysis or enzymatic hydrolysis, and by subsequent GC and GC-MS analyses. The monoterpene glycoside fraction was then purified by HPLC (ODS, 40–80% MeOH and 20–60% MeCN) to give two glycosides, 1 (12 mg, geranyl 6-O-β-d-xylpyranosyl-β-d-glucopyranoside, \( t_{R} = 55.0 \text{ min} \)) and 2 (5 mg, a linalyl glycoside, \( t_{R} = 52.5 \text{ min} \). [\( \Delta^{2} \)] 54.6 (c 3.20, MeOH); NMR δH (400 MHz, CDCl₃): 1.33 (3H, s, H-10), 1.62 (3H, s, H-9), 1.70 (3H, s, H-8), 1.55–1.75 (2H, br, H-4), 1.90–2.10 (2H, br, H-5), 2.32 (dd, \( J = 8.0, 8.0 \text{ Hz} \), H-2'), 2.35–2.36 (2H, H-2', 5a'), 3.41–3.50 (3H, H-3', 3', 4'), 5.33 (m, H-5'), 3.63 (m, H-4'), 3.82 (dd, \( J = 5.3, 11.9 \text{ Hz} \), H-6a), 3.97 (dd, \( J = 5.4, 11.2 \text{ Hz} \), H-5b'), 4.09 (d, \( J = 9.9 \text{ Hz} \), H-6b), 4.45 (d, \( J = 7.6 \text{ Hz} \), H-1'), 4.54 (d, \( J = 7.9 \text{ Hz} \), H-1'), 5.21 (br, t, \( J = 6.0 \text{ Hz} \), H-6), 5.30 (d, \( J = 17.2 \text{ Hz} \), H-1a), 5.31 (d, \( J = 11.0 \text{ Hz} \), H-1b), 5.89 (dd, \( J = 17.2, 11.0 \text{ Hz} \), H-2) [\( \delta_{D} \)] (see the Table).

Linalyl glycoside 2 was found to have the molecular formula C₁₂H₁₇O₁₀Na by HRFABMS analysis (Pos., NOBA, m/z 471.2211, [M+Na⁺]⁺, +0.5 mmu for C₁₂H₁₈O₁₀Na). In the 13C-NMR spectrum (CD₂OD, see the Table), the signals due to linalyl and glucosyl moieties coincided completely with those of (S)-linalyl 6-O-β-xylpyranosyl-β-d-glucopyranoside (5), and the 11 sugar carbon signals, including two anomic ones, were very similar to those of 1, except for C-1°, suggesting the sugar moiety of 2 to be identical with that of 1.

A comprehensive 1H-NMR spectral analysis, including 1H-1H COSY measurements of the hexaacetate (2a) of 2 facilitated a complete assignment of the proton signals. 2a: NMR δH (400 MHz, CDCl₃): 1.33 (3H, s, H-10), 1.45–1.62 (2H, m, H-4'), 1.57 (3H, s, H-9), 1.66 (3H, s, H-8), 1.85–2.02 (2H, m, H-5), 1.99 (3H, s, 2.02 (3H, s), 2.03 (3H, s), 2.05 (9H, s), 3.34 (dd, \( J = 11.9, 8.6 \text{ Hz} \), H-5'a), 3.58 (dd, \( J = 7.0, 7.0 \text{ Hz} \), H-6'a), 3.60 (m, H-5'), 3.77 (m, H-6b), 4.12 (dd, \( J = 11.9, 4.9 \text{ Hz} \), H-5'b), 4.35 (dd, \( J = 6.6 \text{ Hz} \), H-1'), 4.36 (dd, \( J = 8.1 \text{ Hz} \), H-1'), 4.88 (dd, \( J = 9.5, 9.5 \text{ Hz} \), H-4'), 4.89 (dd, \( J = 8.4, 6.6 \text{ Hz} \), H-2'), 4.93 (dd, \( J = 8.6, 8.4 \text{ Hz} \), H-4'), 4.95 (dd, \( J = 9.5, 8.1 \text{ Hz} \), H-2'), 5.04 (br, t, \( J = 7.0 \text{ Hz} \), H-6), 5.12 (dd, \( J = 8.4, 8.4 \text{ Hz} \), H-3'), 5.16 (dd, \( J = 9.5, 9.5 \text{ Hz} \), H-3), 5.20 (dd, \( J = 17.6, 1.1 \text{ Hz} \), H-1a), 5.27 (dd, \( J = 11.0, 1.1 \text{ Hz} \), H-1b), 5.71 (dd, \( J = 17.6, 11.0 \text{ Hz} \), H-2'), 6.6-a and 6'b did not show a significant acetylation shift, indicating that the xylose group was linked to C-6'. The presence of two sets of sequential trans-1,2-diauxial relationships (\( J = 8.8–11 \text{ Hz} \) in H-1° and H-1') confirmed the sugar moiety of 2 to be identical with that of 1.

The coincidence of the 13C-NMR signals of the linalyl group of 2 with those of 5 suggested the absolute configuration of the linalyl group of 2 to be S. A comparison of the 13C-NMR data (D₂O) of 2 with those of diastereomeric linalyl glycosides 6R and 6S* (see the Table; different standards resulted in different chemical shifts (Δδ 2.0–2.2)) also indicated the absolute configuration of the linalyl moiety of 2 to be S. This was confirmed by a chiral GC analysis in a chiral column of CP-cyclodex B-236M (standard internal of ethyl decanoate, \( t_{R} = 71.9 \text{ min} \) of the linalool released from 2 by an enzymatic hydrolysis with acetone powder prepared from fresh tea leaves. The linalool from 2 was detected at relative \( t_{R} = 0.845 [\text{1S-isomer}] \) clearly separated from that of the authentic (R)-isomer (relative \( t_{R} = 0.840 \)). No (R)-isomer was detected. Thus, 2 was unambiguously determined to be (S)-linalyl 6-O-β-xylpyranosyl-β-d-glucopyranoside [= (S)-linalyl β-primeveroside, see the Fig.]. Linalyl β-primeveroside has been isolated from the herb *Cynanchum hancockianum*, although its absolute structure remained unknown. After comparing with our data, the linalyl β-primeveroside from *C. hancockianum* was proved to be (R)-linalyl β-primeveroside, a diastereomer of 2.

The aroma precursors of 2-phenylethanol and benzyl alcohol were isolated from another cultivar (Maoxie) for oolong tea. A

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* Studies on the Aroma Formation Mechanism of Oolong Tea. Part II. For Part I, see ref. 5.
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* Sodium 3-(trimethylsilyl)propionate-2,2,3,3-d₄ (an internal standard).
**Table**  
13C-NMR Data for Monoterpene and Aromatic Alcohol Glycosides

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<th></th>
<th>1 (CD$_2$OD) (100 MHz) (TMS)</th>
<th>2 (CD$_2$OD) (100 MHz) (TSP)</th>
<th>2 (C$_2$D$_2$N) (100 MHz) (TMS)</th>
<th>2 (CD$_2$OD) (100 MHz) (TMS)</th>
<th>2a (CDCl$_3$) (67.8 MHz) (TMS)</th>
<th>3 (CD$_2$OD) (50 MHz) (TMS)</th>
<th>4 (CD$_2$OD) (50 MHz) (TMS)</th>
<th>5 (CD$_2$OD) (50 MHz) (TMS)</th>
<th>6R (D$_2$O) (50 MHz) (TMS)</th>
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<td>23.3</td>
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</table>

C-1': 100.3 | 100.1 | 99.5 | 99.7 | 99.6 | 96.0 | 104.5 | 103.4 | 99.6 | 97.8 | 98.0 |

C-1'': 105.6 | 106.2 | 105.3 | 105.8 | 105.9 | 100.2 | 105.6 | 105.7 | 104.6 | 108.9 | 108.9 |

$\delta$ Chemical shifts with the same superscripts may be vertically interconvertible.

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a, b Chemical shifts with the same superscripts may be horizontally interconvertible.

2. (S)-Linalyl glycoside from oolong tea; 2a: hexaacetate of 2; other signals (6 x acetyl) $\delta$ 20.7 (x 2), 20.8 (x 4), 169.1, 169.3, 169.6, 169.9, 170.1, and 170.4.

2a: Linalyl 6-O-β-D-glucopyranosyl-β-d-glucopyranoside from Cynanchum hancockianum.  
3. 2-Phenylethyl glycoside from oolong tea.

4. Benzyl glycoside from oolong tea.

5. (S)-Linalyl 6-O-β-D-glucopyranosyl-β-D-glucopyranoside.  
6R: (R)-Linalyl 6-O-β-D-arabinofuranosyl-β-D-glucopyranoside.  
6S: (S)-Linalyl 6-O-α-D-arabinofuranosyl-β-D-glucopyranoside.

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**Fig.** Structures of (S)-Linalyl, 2-Phenylethyl, and Benzyl β-Primeverosides.

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2: R=H; R'=Lin  
3: R=H; R'=2-PhE  
2a: R=Ac; R'=Lin  
4: R=H; R=Bz

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hot-water extract of the dried leaves (2 kg) was chromatographed on active charcoal (H$_2$O–MeOH) and then on Amberlite XAD-2 (H$_2$O–MeOH). The fraction containing the precursors of 2-phenylethanol and benzyl alcohol was passed through a Polymeric AT column to remove the catechins, and then chromatographed on Sephadex LH-20 (50% MeOH) and ODS (40%–100% MeOH). The 60% and 40% MeOH fractions were purified by HPLC (ODS, H$_2$O–MeCN) to give 2-phenylethyl β-primeveroside [3, ca. 10 mg; [α]$_{233}^{23}$ = 52.9 ($c$ 0.42, MeOH)] and benzyl β-primeveroside [4, ca. 12 mg; [α]$_{233}^{23}$ = 69.5 ($c$ 0.50, MeOH)], respectively. The structures of 3 and 4 were determined in a similar manner to that for 2 (13C-NMR, see the Table).

2-Phenylethyl and benzyl β-primeverosides have been isolated from Alangium plataniolium var. trilobum and in the dried roots of Rehmannia glutinosa Libosch, var. purpurea Makino. Benzyl β-primeveroside has also been found in the green fruits of Prunus laurocerasus, as well as in a culture of Panax ginseng, etc.  

These glycosides were, to our knowledge, discovered for the first time as aroma precursors by our study. The result that aroma precursors 1, 2, 3, and 4 from oolong tea are primeverosides may imply the specific formation mechanism of alcoholic aroma constituents in oolong tea. This also raises an interesting point about the origin of tea plants (alcoholic aromas are produced from β-primeverosides, not from glucosides). Studies on the distribution of these aroma precursors in tea plants and on the enzymes concerned with aroma formation are now in progress.

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leaves to prepare the crude enzyme.

References