Note

(Z)-3-Hexenyl-β-D-glucopyranoside in Fresh Tea Leaves as a Precursor of Green Odor

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The glycoside fraction from fresh tea leaves was acetylated and separated by silica gel column chromatography. A crystalline product was identified as (Z)-3-hexenyl-(tetra-O-acetyl)-β-D-glucopyranoside from spectrometric data which were identical with those of an authentic synthesized sample in all respects.

There are two possible processes for the formation of the greenish odor of plant materials, these being a biosynthetic process from the lipid and enzymatic hydrolysis of (Z)-3-hexenyl-β-D-glucoside.

(Z)-3-Hexenol (leaf alcohol) is the main constituent contributing to the characteristic greenish odor in vegetables and teas. The biosynthetic route to (Z)-3-hexenol from linolenates with lipoxigenase and lyase has been clarified by Hatanaka et al. According to this biosynthetic route, the first decomposition product of linolate hydroperoxide with lyase is (Z)-3-hexenal, which is isomerized to (E)-2-hexenal, a leaf aldehyde, or is reduced to (Z)-3-hexenol. However, Saijo and Takeo and Yamanishi et al. have pointed out that, during the manufacture of black tea, the content of (Z)-3-hexenol is highest in the early stage of the manufacturing process and, with the increase of enzymatic activity in the crushed tea leaves, the content of (E)-2-hexenal increases with the decrease of (Z)-3-hexenol. This means that there could be another formation mechanism for these aroma constituents of black tea along the biosynthetic route from linolate to C6-alcohol and aldehyde. This hypothesis led us to search for an aroma precursor that would directly produce (Z)-3-hexenol.

In our previous report, (Z)-3-hexenol and benzyl alcohol were identified, together with some monoterpenes alcohols, from the glycosidic fraction of green tea after hydrolyzing with acetone power prepared from fresh tea leaves. Later, benzyl-β-D-glucoside was separated and identified in the glycosidic fraction as its acetate derivative. This is the first identification of a glycoside in tea leaves as a precursor of the black tea aroma.

In our preceding study, as well as tetraacetylated benzyl-β-D-glucopyranoside, another single compound was obtained in an eluate a little earlier than that of the benzyl derivative. This eluate was crystallized and recrystallized from ether-pentane (3:1) as a white leaflet with mp 63°C. The IR spectrum indicated a double bond (3010, 1640 cm⁻¹), and the lack of a 960 cm⁻¹ absorption band suggests a (Z)-structure. EIMS (70 eV) showed the molecular ion at m/z 430, typical fragment ions of acetylated glucose at m/z 331, 169, 157, and 109, and of the hexenol moiety at m/z 82, 69, 67, and 55. In the PMR (270 MHz) spectrum, a doublet (J = 8.06 Hz) at δ 4.52 established the β-configuration for the glucoside. The signals at δ 0.95, 2.00-2.12, and 2.29-2.36 explain the methyl and methylene protons of a hexenol moiety, and two protons on C-1 showed two multiplets at 3.44-3.53 and 3.83-3.92, respectively. CMR data showed six signals that could be assigned to six carbons in a 3-hexenyl moiety, except that the C-5 carbon overlapped the acetyl-C signal. All spectrometric data are summarized in Table. The foregoing results enabled the structure of this glucoside to be assigned as

Table: Spectral Data for (Z)-3-Hexenyl-(tetra-O-acetyl)-β-D-glucoside

| IR  | 3010, 1740, 1640, 1220 |
| MS  | 430 (M⁺), 331 (33), 245 (6), 229 (4), 169 (100), 157 (27), 139 (11), 109 (43), 82 (78), 69 (11), 67 (81), 55 (10), |
| PMR | 0.95 (3H, t, J = 7.57 Hz), 2.01 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 2.00-2.12 (2H, m), 2.29-2.36 (2H, m), 3.44-3.53 (1H, m), 3.67-3.73 (1H, m), 3.83-3.92 (1H, m), 4.14 (1H, dd, J = 2.45, 12.33 Hz), 4.27 (1H, dd, J = 4.76, 12.21), 4.52 (1H, d, J = 8.06 Hz), 4.99 (1H, t, J = 5.86 Hz), 5.09 (1H, t, J = 6.35 Hz), 5.21 (1H, t, J = 6.27 Hz), 5.26-5.33 (1H, m), 5.42-5.48 (1H, m), |
| CMR | 14.23, 20.65, 20.68, 20.75, 27.61, 62.06, 68.56, 69.87, 71.36, 71.85, 72.92, 100.91, 124.16, 134.11, 169.32, 169.43, 170.35, 170.71 |

*cm⁻¹ as KBr microcrystals.

m/z (rel. int. %) at 70 eV.

δ value in CDCl₃.

Fig. Structure of (Z)-3-Hexenyl-(tetra-O-acetyl)-β-D-glucopyranoside.

(Z)-3-hexenyl-(tetra-O-acetyl)-β-D-glucoside (Fig.), which was synthesized from acetobromoglucose and (Z)-3-hexenol by a modified Koenig-Knorr reaction. The natural synthesized sample was identical with the isolated sample in all spectrometric data. These results suggest (Z)-3-hexenol to be present as its glucoside in fresh tea leaves, and that the formation of this alcohol at the early stage of black tea manufacture is from enzymatic hydrolysis of the glucoside precursor.

Nagumo et al. have separated the same glucoside from Pertya globrescens Sch. Bip. and elucidated the same structure, however, they did not note that this glucoside would be an important flavor precursor of food products from various plant origins.

The greenish odor of plant material which is caused by C6-alcohols, aldehydes and esters has been studied by both food chemists and phytochemists. It should be recognized that there are two possible formation processes for these compounds: a biosynthetic process from the lipid, and enzymatic hydrolysis of the glycoside.

References
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