Note

Structure of Sesquiterpenoid
Glucoside from Flue-cured
Tobacco LeavesHiroyuki TAZAKI, Hisashi KODAMA,
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Several sesquiterpenoids have been reported as phytoalexins of Solanaceae, whose structures are closely related to each other. We have previously reported twelve terpene glycosides from tobacco leaves.¹⁻⁵ In this paper, sesquiterpenoid glucoside a newly identified as a tobacco constituent will be presented.

The isolation procedure was the same as that described in the previous paper.¹ After several steps involving extraction and chromatography, the 30% MeOH fraction (1074 mg) was obtained by column chromatography (silica-gel CHCl₃-MeOH) and further separated by HPLC, using columns of YMC S343 and YMC A314. The fraction positive to the glucosidase assay² was collected and acetylated, affording compound **1** (35 mg) by using a column of AM324.

Compound **1** was obtained as a colorless viscous substance, and the FD-MS data showed the parent peak of *m/z* 584 and a characteristic fragment peak of *m/z* 331 as a tetraacetylated hexose unit. This indicates that **1** had a tetra-acetyl hexopyranose as the sugar moiety and that the molecular weight of the aglycone was 254. ¹H-NMR spectral data for **1** showed the presence of one methyl (δ 0.92) joined to a methine, two singlet methyls (δ 1.04, 1.22) and five acetyl methyls (δ 1.98–2.07). One of the acetyl groups existed in aglycone moiety. Ring protons of the sugar moiety (δ 3.40–5.18) were assigned to axial protons by their coupling constants ($J=7.9$ –9.9 Hz). The coupling

constant of the anomeric proton (δ 4.50, $J=7.9$ Hz) indicated that **1** was an acetylated β -glucoside. Compound **1** was then saponified with NH₃-MeOH to give the free glycoside, and β -glucosidase successfully hydrolyzed this free glycoside to liberate the aglycone. The aglycone had the molecular formula of C₁₅H₂₆O₃, which was determined from the high-resolution mass spectrum (HR-MS). The ¹H-NMR spectral data for the aglycone were similar to those for 1 β -hydroxydebneyol (**3**), which is an antifungal compound that was elicited from a cell suspension culture of *Nicotiana tabacum*.⁶ The signals of the aglycone at δ 0.94, 1.10, 1.36, 3.40, 3.56, 4.23 and 5.81 were identical with the signals of **3**. The ¹H-¹H COSY experiment results for **3** permitted the assignment of all proton signals. The spin systems of (H-1)-(H-2 α , β)-(H-3 α , β)-(H-4)-H(14) and (H-6 α , β)-(H-7)-(H-8 α , β)-(H-9) were verified by the cross peaks. To ascertain the stereochemistry of **3**, a nuclear Overhauser enhancement (NOE) difference spectrum was obtained. Irradiation of the C-15 methyl group (δ 1.36) caused enhancement of the signals at δ 1.72, 1.73 and 2.34; these signals were due to protons H-8 β , H-6 β and H-3 β . The low-field H-3 β resonance suggested a 1-3 *cis*-axial interaction between H-3 β and the 1-hydroxy group, with H-1 having an α -configuration. Irradiation of the C-14 methyl group (δ 0.94) caused enhancements of the signals at δ 1.28, 1.65 and 1.75; these signals were due to protons H-6 α , H-2 α and H-7 α . Therefore, the aglycone was identified as 1 β -hydroxydebneyol (**3**). Aglycone **3** had three hydroxy groups, and a comparison of the NMR signals of **1** and **3** confirmed the acetylation and glucosylation site of **1**. The low-field H-1 resonance at δ 5.22 in **1** strongly suggested C-1 as the acetylation site, and the low-field C-12 resonance at δ 78.0 in **1** strongly suggested C-12 as the glucosylation site. From these observations, the original glucoside was identified as 1 β -hydroxydebneyol-12-*O*- β -D-glucopyranoside (**2**).

Recently, debneyol and its related compounds have been isolated from a cell suspension culture of *N. tabacum*^{6,7} and virus inoculated leaves of *N. debneyi*.^{8,9} 1 β -Hydroxydebneyol (**3**) and its glucoside **2** are additions to the small number of known *trans*-dimethyletheromorphilanes.

Experimental

Enzymic hydrolysis. β -Glucosidase (from sweet almond,

1 : R¹=Ac, R²=GlcAc

2 : R¹=H, R²=Glc

3 : R¹=R²=H

Glc : β -glucopyranosyl

Ac : acetyl

P.L. Biochemicals Inc.) had no activity to α -benzyl glucosides. The Glucoside was suspended in 0.1 M sodium acetate buffer (pH 5.25), and β -glucosidase was added at 38°C. After 12 hr, the aglycone was extracted from the solution with ether.

High-performance liquid chromatography. HPLC was performed on reversed phase columns, YMC S343 (25 cm $\times$ 20 mm i.d.), YMC A314 (30 cm $\times$ 6.0 mm i.d.) and YMC AM 324 (30 cm $\times$ 10 mm i.d.), with MeOH-H₂O solvent system, using a Hitachi 655 constant flow pump and a Shodex SE-31 differential refractometer to monitor the column effluent.

Instrumental analysis. Mass spectra were measured by a Hitachi M-80 instrument, and NMR spectra were obtained with a Bruker AM 500. Chemical shifts are expressed as ppm downfield from TMS as the internal standard. A two dimensional NMR experiment was performed on the Bruker AM 500 using available 2D software.

Isolation of the glucoside tetraacetate (1). The isolation procedure from 4.7 kg of flue-cured tobacco leaves (*N. tabacum* L., OCL) was the same as that described in the previous paper.

1 β -hydroxydebneyol-12-O- β -D-glucopyranoside tetraacetate (1). Yield, 35 mg. ¹H-NMR (CDCl₃) δ : 0.92 (3H, d, J =7.1 Hz, H-14), 1.04 (3H, s, H-13), 1.19 (2H, m), 1.22 (3H, s, H-15), 1.55 (1H, m), 1.72 (3H, m), 1.98 (6H, s), 2.00 (3H, s), 2.01 (3H, s), 2.07 (3H, s), 1.91–2.24 (4H, m), 3.40 (1H, d, J =9.9 Hz, H-12), 3.69 (1H, ddd, J =2.4, 5.1, 9.5 Hz, H-5'), 3.77 (1H, d, J =9.9 Hz, H-12), 4.13 (1H, dd, J =2.4, 12.2 Hz, H-6'), 4.21 (1H, dd, J =5.1, 12.2 Hz, H-6'), 4.50 (1H, d, J =7.9 Hz, H-1'), 4.99 (1H, dd, J =7.9, 9.7 Hz, H-2'), 5.05 (1H, dd, J =9.7, 9.7 Hz, H-3'), 5.18 (1H, dd, J =9.5, 9.7 Hz, H-4'), 5.22 (1H, br. t, J =2.7 Hz, H-1), 5.93 (1H, d, J =6.5 Hz, H-9). ¹³C-NMR (CDCl₃) δ : 17.0 (C-14), 20.4 (C-13), 20.6 (CH₃), 21.7 (CH₃ $\times$ 2), 22.6 (CH₃ $\times$ 2), 25.5 (CH₂), 26.1 (CH₂), 26.4 (CH₂), 31.8 (C-15), 38.1 (C-5), 38.9 (CH₂), 39.5 (CH), 40.9 (C-4), 61.9 (C-6'), 68.4 (C-2'), 71.4 (C-4'), 71.8 (C-5'), 72.7 (C-3'), 73.3 (C-11), 76.2 (C-1), 78.0 (C-12), 101.4 (C-1'), 129.6 (C-9), 137.5 (C-10), 169.4 (C $\times$ 2), 170.2 (C), 170.6 (C $\times$ 2).

Saponification and enzymic hydrolysis of 1. Saponification of **1** (20 mg) was performed with saturated NH₃-MeOH at room temperature for 12 hr. The reaction mixture was concentrated by evaporation and then submitted to enzymic hydrolysis to yield 1 β -hydroxydebneyol (**2**, 7 mg). ¹H-NMR (CDCl₃) δ : 0.94 (3H, d, J =7.1 Hz, H-14), 1.10 (3H, s, H-13), 1.17 (1H, m, J =2.1, 4.4, 13.8 Hz, H-3 α), 1.28 (1H, m, H-6 α), 1.36 (3H, s, H-15), 1.60 (1H, m, J =2.1, 7.1 Hz, H-4), 1.65 (2H, m, H-2), 1.72 (1H, m, H-8 β), 1.73 (1H, m, H-6 β), 1.75 (1H, m, H-7 α), 1.99 (1H, m, J =3.4, 7.0, 5.5 Hz, H-8 α), 2.34 (1H, m, J =4.6, 13.8 Hz, H-3 β), 3.40 (1H, br. d, H-12), 3.56 (1H, br. d, H-12), 4.23 (1H, br. s, H-1), 5.81 (1H, dd, J =1.6, 7.0 Hz, H-9). ¹³C-NMR (CDCl₃) δ : 16.8 (C-14), 20.3 (C-13), 24.9 (C-2), 26.6 (C-8), 28.1 (C-3), 33.0 (C-15), 38.2 (C-5), 39.3 (C-6), 39.6 (C-7), 41.4 (C-4), 68.5 (C-12), 74.4 (C-11), 74.7 (C-1), 126.8 (C-9), 142.3 (C-10). EI-MS m/z (rel. int.): 254 (M⁺, 1), 236 (2), 223 (4), 218 (5), 205 (8), 177 (6), 161 (10), 147 (8), 119 (20), 105 (40), 91 (30), 57 (28), 43 (100). HR-MS m/z : 254.1903; 254.1882 calcd. for C₁₅H₂₆O₃.

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