Glycosidic Constituents from in Vitro Anoectochilus formosanus

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The glycosidic constituents of whole plants of Anoectochilus formosanus propagated by tissue culture were investigated. A new compound, 2-(β-D-glucopyranosylmethyl)-5-hydroxymethylfuran, along with the known compounds, 3-(R)-3-β-D-glucopyranosylobutanal (kingsenose), 3-(R)-3-β-D-glucopyranosylxy-4-hydroxybutanoic acid, 1-O-isopropyl-β-D-glucopyranoside, (R)-(−)-3,4-dihydroxybutanoic acid γ-lactone, 4-(β-D-glucopyranosyloxy)benzyl alcohol, (6R,9S)-9-hydroxy-megastigma-4,7-dien-3-one-9-α-D-glucopyranoside, and corticosteroid C were isolated.

Key words Anoectochilus formosanus; tissue culture; glycoside

Anoectochilus formosanus Hayata is an orchidaceous perennial herb distributed only in Taiwan (China) and Okinawa (Japan). Its whole plant has been used since ancient times as a folk medicine for the treatment of fever, pleurisy, snake-bite, lung disease, and underdeveloped children in Taiwan. Recently, this herbal drug was also used to cure hypertension, diabetes mellitus, consumption and nephritis, etc. Since the natural sources of A. formosanus have been largely depleted, we started to investigate the propagation of this species by tissue culture techniques, and worked on its chemical components and pharmacological profiles. In the present paper, we report here the further isolation and characterization of glycosidic constituents from the in vitro whole plants of cultured A. formosanus.

The dried whole plants cultured were extracted with MeOH at room temperature, and the concentrate was suspended in H2O and partitioned successively with CHCl3 and n-BuOH. The residues obtained from the H2O layer and the n-BuOH layer were separately subjected to normal-phase and reversed-phase silica gel column chromatography, then preparative TLC to give compounds 1–10. The structures of the known compounds were identified as glucose (1), sucrose (2), 3-(R)-3-β-D-glucopyranosylobutanal (3), 3-(R)-3β-D-glucopyranosyloxy-4-hydroxybutanoic acid (4), 1-O-isopropyl-β-D-glucopyranoside (5), (R)-(−)-3,4-dihydroxybutanoic acid γ-lactone (6), 4-(β-D-glucopyranosyloxy)benzyl alcohol (6), (6R,9S)-9-hydroxy-megastigma-4,7-dien-3-one-9-α-D-glucopyranoside (8), and corticosteroid C (9) by comparing their spectral data with those previously reported.

Compound 7 gave a molecular ion at m/z 290 [M]+ in EI mass spectrometry, and high-resolution EI-MS revealed the molecular formula C12H18O8 [M]+ 290.0997, Caled 290.1002. In the 1H- and 13C-NMR spectra of 7, the signals at δC 57.5 [CH3], δH 4.48 (2H, s), δC 63.7 [CH2], δH 4.63 (1H, d, J=12.8 Hz), 4.79 (1H, d, J=12.8 Hz), δC 109.2 [CH, δH 6.25 (1H, d, J=3.3 Hz)], δC 111.7 [CH, δH 6.36 (1H, d, J=3.3 Hz)], δC 152.4 [C], and δC 156.5 [C] revealed the presence of a 2,5-dihydroxyethylmethyl group. On complete acidic hydrolysis, 7 gave D-glucose. The absolute configuration of the sugar was defined by GC analysis using the Hara method. In the 1H- and 13C-NMR spectra, anomeric signals were observed at δH 4.34 (1H, d, J=7.9 Hz) and δC 102.9, as a β-linkage of D-glucose. The NOESY spectrum of 7 showed a cross peak between δH 4.34 (H-1′ of Glc) and δH 4.63 (H-6b), indicating that glucose unit is linked to the 5-hydroxymethyl of the furan ring (Fig. 1). In the correlation spectroscopy via long-range coupling (COLOC) spectrum of 7...
(Fig. 1), the long-range correlation was observed from the $\delta_H$ 4.63 (H-6b) and 4.79 (H-6a) to the $\delta_C$ 102.9 (C-1 of Glc), supporting the above deduction. Thus, 7 was determined to be 2-(β-D-glucopyranosylxymethyl)-5-hydroxymethylfluran.

The new furanoid glycoside (7) was assayed for several pharmacological activities such as the inhibition of $\alpha$-glucosidase, and interaction with rat adipocytes, however, no activity was found. Compound 10 was only isolated from the dried plant, suggesting that 10 is produced as an artificial product, because 10 was not found in the fresh plants of A. formosanus and A. koshunensis. This is one reason the fresh plants are widely used as the Chinese folk remedy.

Experimental

**General Procedures** 1H- and 13C-NMR were measured in CD$_2$OD or pyridine-d$_6$ on a JEOL 270 instrument, using TMS as an internal standard. Analytical GC was carried out on a Hitachi G-3000 gas chromatograph. Melting points were determined with a YAZAWA BV-2 apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1010 digital polarimeter.

**Micropropagation System of A. formosanus** The ripened fruits of A. formosanus (collected in Taiwan province) were sterilized with 1% (v/v) of NaOCl containing 0.05% Tween 20 for 15 min, then with 70% (v/v) of ethanol for 30 s, and washed with sterilized H$_2$O 3 times. The fruits were dissected longitudinally and seeds were cultured on 1/2 MS liquid medium supplemented with 1 mg/l 6-benzylaminopurine (BA) and 2 g/l peptone. The seeds were germinated at 25°C under 1500 lux for 16 h per day by fluorescent propagation light. After 30 days the seeds germinated and formed hypocotyl. Afterwards, the seedlings, 6—8 mm in length, were transferred to 1/2 MS liquid medium to produce the multiple shoot complex. They were cultured in 1/2 MS liquid medium supplemented with 0.3 mg/l BA and 0.03 mg/l α-naphthalenacetic acid (NAA) under shaking at 30 rpm. After 2 months they were transferred to 1/2 MS liquid medium without growth regulator and shaken for 4 more months. The regenerated plantslets were transplanted into a soil mixture for 6—8 months to grow until they reached 15—18 cm in height and 2.8—3.3 g in weight per plantlet. A voucher specimen has been deposited at the Herbarium of the Medicinal Plant Garden of Pharmaceutical Sciences, Kyushu University.

**Extraction and Isolation** The dried whole plants of cultivated A. formosanus (2.3 kg) were powdered and percolated with MeOH at room temperature, and the concentrate (695 g) was partitioned successively between CHCl$_3$ and H$_2$O, n-BuOH and H$_2$O. The residue obtained from the H$_2$O-soluble portion (576 g) was subjected to an ODS (Cosmosil 75C$_5$-OPN) column eluted with H$_2$O to give four fractions. A small part of the fr. 1 (0.1 g), and a small part of the fr. 2 (0.1 g) were chromatographed on silica gel (CHCl$_3$–MeOH–H$_2$O, 6:4:1) to afford sucrose (23 mg) and glucose (38 mg), respectively. A part of the fr. 3 (1 g) was chromatographed on silica gel eluted with a gradient of CHCl$_3$–EtOH (8 : 3 : 7 : 5) to give 3 (780 mg). Purification of fr. 3 (80 mg) by preparative TLC (Kieselgel 6 F$_254$, 1 mm, Merck) with CHCl$_3$–MeOH–H$_2$O (6 : 4 : 1) gave 4 (17 mg). The fr. 4 (1.8 g) was purified by silica gel chromatography (CHCl$_3$–MeOH–H$_2$O, 7 : 3 : 0.1) to give 5 (970 mg) and 10 (12 mg). The residue obtained from the n-BuOH portion (59 g) was subjected to Diaion HP-20 (H$_2$O–MeOH, 90:10—0:100), Cosmosil 75C$_5$–OPN (MeOH–H$_2$O, 20:80—95:5) and silica gel (CHCl$_3$–MeOH–H$_2$O, 7:3:0.5) column chromatographies to provide compounds 6 (73 mg), 7 (59 mg), 8 (22 mg), and 9 (18 mg).

2-(β-D-Glucopyranosylxymethyl)-5-hydroxymethylfluran (7) A colorless amorphous powder. [α]$_D$ = −73.5° (c = 1.3, MeOH). EI-MS m/z 290 [M]$^+$; HR-EI-MS m/z 290.0997 ([M]$^+$, Calcd for C$_{12}$H$_{20}$O$_{5}$ 290.1002). 1H-NMR (DMSO-d$_6$): δ 6.36 (1H, d, J = 3.5 Hz, H-4), 6.25 (1H, d, J = 3.3 Hz, H-3), 4.79 (1H, d, J = 12.8 Hz, H-6a), 4.63 (1H, d, J = 12.8 Hz, H-6b), 4.48 (2H, s, H-7), 4.34 (1H, d, J = 7.9 Hz, H-1'), 3.87 (1H, d, J = 11.9, 2.1 Hz, H-6'), 3.67 (1H, dd, J = 11.9, 5.5 Hz, H-6'), 3.34 (1H, m, H-3'), 3.31 (1H, m, H-4'), 3.26 (1H, m, H-5'), 3.19 (1H, dd, J = 7.9, 9.0 Hz, H-2'). 13C-NMR (CD$_2$OD): δ: 156.5 (C-2), 109.2 (C-3), 111.7 (C-4), 152.4 (C-5'), 63.7 (C-6'), 57.5 (C- 7), 102.9 (C-1'), 75.1 (C-2'), 78.1 (C-3'), 71.7 (C-4'), 78.1 (C-5'), 62.9 (C- 6').  

**Acidic Hydrolysis of 7** Compound 7 (3 mg) was hydrolyzed with 1 M H$_2$SO$_4$ (1 ml) under 95°C for 1 h. The reaction mixture was diluted with H$_2$O and extracted with CHCl$_3$. The aqueous layer was neutralized with Ba(OH)$_2$, then filtered. The filtrate was concentrated, and the residue was purified by Sephadex LH-20 column chromatography (MeOH) to afford a sugar fraction. The sugar sample was subjected to GC analysis after conversion into the TMSI ether of methyl thiazolidine-4 (R-carboxylate derivatives according to Hara et al. ([condition: GL Sciences OV-17, 50 m x 0.25 mm i.d.; isothermal 220°C, He at 1.5 kg cm$^{-2}$]; t$_{R}$ (min): 27.08 (ε-glucose).

References