Glycosidic Constituents from *in Vitro* *Anoectochilus formosanus*

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Received May 24, 2000; accepted July 18, 2000

The glycosidic constituents of whole plants of *Anoectochilus formosanus* propagated by tissue culture were investigated. A new compound, 2-(β -D-glucopyranosyloxymethyl)-5-hydroxymethylfuran, along with the known compounds, 3-(*R*)-3- β -D-glucopyranosyloxybutanolide (kinsenocide), 3-(*R*)-3- β -D-glucopyranosyloxy-4-hydroxybutanoic acid, 1-*O*-isopropyl- β -D-glucopyranoside, (*R*)-(+)-3,4-dihydroxybutanoic acid γ -lactone, 4-(β -D-glucopyranosyloxy)benzyl alcohol, (6*R*,9*S*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*- β -D-glucopyranoside, and corchoionoside 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, pleurodynia, snake-bite, lung disease, and underdeveloped children in Taiwan.¹⁾ Recently, this herbal drug was also used to cure hypertension, diabetes mellitus, consumption and nephritis, etc.²⁾ 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.^{3–6)} 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-glucopyranosyloxybutanolide (3),^{3,5,7)} 3-(*R*)-3- β -D-glucopyranosyloxy-4-hydroxybutanoic acid (4),^{3,5)} 1-

O-isopropyl- β -D-glucopyranoside (5),³⁾ (*R*)-(+)-3,4-dihydroxybutanoic acid γ -lactone (10),⁷⁾ 4-(β -D-glucopyranosyloxy)benzyl alcohol (6),⁸⁾ (6*R*,9*S*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*- β -D-glucopyranoside (8),⁹⁾ and corchoionoside C (9),¹⁰⁾ 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, Calcd 290.1002). In the ¹H- and ¹³C-NMR spectra of 7, the signals at δ_C 57.5 [CH₂, δ_H 4.48 (2H, s)], δ_C 63.7 [CH₂, δ_H 4.63 (1H, d, J =12.8 Hz), 4.79 (1H, d, J =12.8 Hz)], δ_C 109.2 [CH, δ_H 6.25 (1H, d, J =3.3 Hz)], δ_C 111.7 [CH, δ_H 6.36 (1H, d, J =3.3 Hz)], δ_C 152.4 [C], and δ_C 156.5 [C] revealed the presence of a 2,5-dihydroxymethylfuran group. On complete acidic hydrolysis, 7 gave D-glucose. The absolute configuration of the sugar was defined by GC analysis using the Hara method.¹¹⁾ In the ¹H- and ¹³C-NMR spectra, anomeric signals were observed at δ_H 4.34 (1H, d, J =7.9 Hz) and δ_C 102.9, as a β -linkage of D-glucose. The NOESY spectrum of 7 showed a cross peak between δ_H 4.34 (H-1' of Glc) and δ_H 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

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Fig. 1. NOE Correlations in NOESY and C-H Long-Range Correlations in COLOC Spectra of **7**

(Fig. 1), the long-range correlation was observed from the δ_{H} 4.63 (H-6b) and 4.79 (H-6a) to the δ_{C} 102.9 (C-1 of Glc), supporting the above deduction. Thus, **7** was determined to be 2-(β -D-glucopyranosyloxymethyl)-5-hydroxymethylfuran.

The new furanoid glycoside (**7**) was assayed for several pharmacological activities such as the inhibition of α -glucosidase, and interaction with rat adipocytes,¹² 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 ^{13}C -NMR were measured in CD_3OD or pyridine- d_5 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 BY-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) EtOH for 30 s, and washed with sterilized H_2O 3 times. The fruits were dissected longitudinally and seeds were cultured on 1/2 MS medium¹³ 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 d the seeds germinated and formed protocorm. 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 -naphthaleneacetic 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 plantlets 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 cultured *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_2O , *n*-BuOH and H_2O . The residue obtained from the H_2O -soluble portion (576 g) was subjected to an ODS (Cosmosil 75C₁₈-OPN) column eluted with H_2O 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_2O , 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 60 F₂₅₄, 1 mm, Merck) with CHCl_3 -MeOH- H_2O (6:4:1) gave **4** (17 mg). The fr. 4 (1.8 g) was purified by silica gel chromatography (CHCl_3 -MeOH- H_2O , 7:3:0.1) to give **5** (970 mg) and **10** (12 mg). The residue obtained from the *n*-BuOH portion (39 g) was subjected to Diaion HP-20 (H_2O -MeOH, 90:10—0:100), Cosmosil 75C₁₈-OPN (MeOH- H_2O , 20:80—95:5) and silica gel (CHCl_3 -MeOH- H_2O , 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-Glucopyranosyloxymethyl)-5-hydroxymethylfuran (7**)** A colorless amorphous powder. $[\alpha]_{\text{D}}^{20} -73.5^\circ$ ($c=1.3$, MeOH). EI-MS m/z 290 $[\text{M}]^+$; HR-EI-MS m/z 290.0997 $[\text{M}]^+$, Calcd for $\text{C}_{12}\text{H}_{18}\text{O}_8$; 290.1002. ^1H -NMR (CD_3OD) δ : 6.36 (1H, d, $J=3.3$ 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, H₂-7), 4.34 (1H, d, $J=7.9$ Hz, H-1'), 3.87 (1H, dd, $J=11.9$, 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'). ^{13}C -NMR (CD_3OD) 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_2SO_4 (1 ml) under 95 °C for 1 h. The reaction mixture was diluted with H_2O and extracted with CHCl_3 . The aqueous layer was neutralized with $\text{Ba}(\text{OH})_2$, 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.*¹¹ (condition: GL Sciences OV-17, 50 m $\times$ 0.25 mm i.d.; isothermal 220 °C; He at 1.5 g cm⁻²; t_{R} (min): 27.08 (D-glucose).

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