



GLUCOSYLATION OF PHENOLICS BY HAIRY ROOT CULTURES OF *LOBELIA SESSILIFOLIA*

MICHIKO YAMANAKA,* KOICHIRO SHIMOMURA,† KAZUO SASAKI,‡ KUNITOSHI YOSHIHARA,‡ and KANJI ISHIMARU*§

*Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, 1 Honjo, Saga 840, Japan; †Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, 1 Hachimandai, Tsukuba, Ibaraki 305, Japan; ‡Division of Applied Sciences, Graduate School of Integrated Science and Art, University of East Asia, 2-1 Ichinomiyu-Gakuen, Shimonoseki 751, Japan

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Abstract—Two new glucosides, (–)-epiafzelechin 7-*O*-β-D-glucopyranoside and protocatechuic acid 3-*O*-β-D-glucopyranoside were isolated from hairy roots of *Lobelia sessilifolia* after cultivation with (–)-epicatechin or protocatechuic acid, respectively.

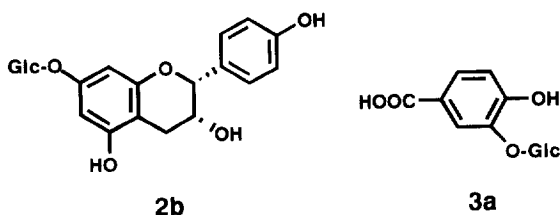
INTRODUCTION

In chemical studies on tissue cultures of Campanulaceous plants, *Lobelia sessilifolia* hairy roots (transformed by *Agrobacterium rhizogenes*) were shown to be rich in glucosylated polyacetylenes [1]. The occurrence of the glucosides in high concentrations in the hairy roots suggested that the roots had a strong capability for glycosylation of secondary metabolites. In the present study, the glucosides biotransformed in the hairy roots cultured in woody plant (WP) medium [2] supplemented with four phenols, (+)-catechin (1), (–)-epicatechin (2), protocatechuic acid (3) or gallic acid (4) as substrates were determined.

RESULTS AND DISCUSSION

Clone A-1 *Lobelia sessilifolia* hairy roots [1] which showed maximum growth in WP liquid medium was selected. From hairy roots cultured in the medium with 1, a monoglucoside compound 1a was isolated, which was identical with (+)-catechin 7-*O*-β-D-glucopyranoside [3]. From hairy roots cultured with 2, two compounds, (–)-epicatechin 7-*O*-β-D-glucopyranoside [3] (2a) and a new glucoside 2b, were isolated.

Compound 2b exhibited a $[M + H]^+$ peak at m/z 437.1459 ($C_{21}H_{25}O_{10}$) in the high-resolution FAB mass spectrum. The 1H and ^{13}C NMR spectra of 2b were similar to those of 2a, showing the presence of a 2,3-*cis*-(epicatechin-type) flavan-3-ol and one glucose



moiety. In the 1H NMR spectrum of 2b, the aromatic proton signals (δ 6.72 and 7.22, each *d*, $J = 8$ Hz) arising from the B-ring suggested that the flavan-3-ol unit of 2b to be (–)-epiafzelechin. The position of the glucose unit was concluded to be at C-7 from the 1H - 1H NOESY spectrum of 2b which showed two correlation cross-peaks between the glucose C-1 proton and the C-6 and C-8 protons. The configuration of the anomeric centre was concluded to be β from the large coupling constant ($J = 8$ Hz) of the glucose C-1 proton signal in the 1H NMR spectrum. Therefore, 2b was characterized as (–)-epiafzelechin 7-*O*-β-D-glucopyranoside.

Hairy roots cultured with 3 produced a new compound, 3a. From its 1H and ^{13}C NMR spectral data, 3a was presumed to be a monoglucoside of 3 which was also confirmed by the $[M - H]^-$ peak at m/z 315.0175 ($C_{13}H_{15}O_9$) in the high-resolution negative SI mass spectrum. The position of the glucose moiety was concluded to be at C-3 from the correlation cross-peak between the glucose C-1 and C-2 protons observed in the 1H - 1H NOESY spectrum of 3a. The configuration of the anomeric centre was concluded to be β from the J -value

§ Author to whom correspondence should be addressed.

(7.4 Hz) of the glucose C-1 proton signal in the ^1H NMR spectrum. Therefore, **3a** was characterized as protocathechuic acid 3-O- β -D-glucopyranoside.

Hairy roots cultured with **4** produced the two known glucosides, β -glucogallin (**4a**) [4] and gallic acid 3-O- β -D-glucopyranoside (**4b**) [5].

Amongst the six compounds (**1a**, **2a**, **2b**, **3a**, **4a** and **4b**) obtained in this experiment, **2b** and **3a** have not been isolated previously from natural plants. Therefore, hairy root cultures of *L. sessilifolia* would appear to be a useful system for the production of new phenol glycosides which could be applicable for the studies of natural chemistry, biochemistry and pharmacognosy.

EXPERIMENTAL

^1H and ^{13}C NMR were measured at 270 and 67.5 MHz, respectively, locked to the major deuterium resonance of the solvent ($(\text{CD}_3)_2\text{CO}$). All media were adjusted to pH 5.7 before autoclaving at 121 for 15 min.

Hairy root cultures. Clone A-1 of *L. sessilifolia* hairy roots was cultured in hormone-free WP liquid medium (50 ml per 100 ml Erlenmeyer flask) in the dark at 25 on a rotary shaker (100 rpm). The substrates (**1** and **2** from Sigma, **3** from Wako and **4** from Nacalai Tesque) were separately dissolved in H_2O and each soln was administered at 2 g l^{-1} (**1**, **2** and **4**) or 0.1 g l^{-1} (**3**) to hairy root cultures which were incubated previously for 4 weeks. After 4 days of culture after administration, hairy roots were harvested.

Isolation of products. (a) **1a**. Lyophilized hairy roots (27.8 g) were mashed and extracted at room temp. for 5 hr with 80% aq. Me_2CO . The extract, after concn under red. pres., was subjected to Sephadex LH-20 ($3.5 \times 30.5\text{ cm}$, 60% aq. MeOH) and Bondapak C18 Porasil B (H_2O -MeOH) CC to give **1a** (140.7 mg). (b) **2a** and **2b**. Using the method described above, **2a** (135.2 mg) and **2b** (5.8 mg) were isolated from the 80% aq. Me_2CO extract of hairy roots (5.1 g, dry wt). (c) **3a** (324.5 mg) was isolated from the MeOH extract of hairy roots (21.5 g, dry wt). (d) **4a** and **4b**. In the same way, **4a** (230 mg) and **4b** (190 mg) were isolated from the 80% aq. Me_2CO extract of hairy roots (8.3 g, dry wt).

(-)-*Epiafzelechin* 7-O- β -D-glucopyranoside (**2b**). Amorphous powder. $[\alpha]_{\text{D}}^{25} = 102.0$ (MeOH, c 0.2). ^1H NMR $[(\text{CD}_3)_2\text{CO} + \text{D}_2\text{O}]$: δ 2.60 (1H, *dd*, $J = 2.0$ and 16.0 Hz, H-4), 2.78 (1H, *dd*, $J = 3.0$ and 16.0 Hz, H-4), 3.30–3.50 (4H, *m*, glc-H-2, 3, 4, 5), 3.60 (1H, *dd*, $J = 12.0$ and 5.0 Hz,

glc-H-6), 3.79 (1H, *dd*, $J = 1.0$ and 12.0 Hz, glc-H-6), 4.12 (1H, *m*, H-3), 4.80 (1H, *d*, $J = 8.0$ Hz, glc-H-1), 4.85 (1H, *br s*, H-2), 6.05 (1H, *d*, $J = 1.0$ Hz, H-8), 6.20 (1H, *d*, $J = 1.0$ Hz, H-6), 6.72 (2H, *d*, $J = 8.0$ Hz, H-3', 5'), 7.22 (2H, *d*, $J = 8.0$ Hz, H-2', 6'). ^{13}C NMR $[(\text{CD}_3)_2\text{CO} + \text{D}_2\text{O}]$: δ 29.1 (C-4), 62.3 (glc-C-6), 66.7 (C-3), 71.0 (glc-C-4), 74.4 (glc-C-2), 77.4 (glc-C-5), 77.5 (glc-C-3), 79.7 (C-2), 97.0 (C-8), 97.5 (C-6), 101.9 (glc-C-1), 102.9 (C-10), 115.8 (C-3', 5'), 129.3 (C-2', 6'), 131.1 (C-1'), 157.0 (C-9), 157.6 (C-5), 157.7 (C-4'), 158.1 (C-7). High-resolution FAB MS m/z (rel. int.): 437.1459 $[\text{M} + \text{H}]^+$ (57).

Protocatechuic acid 3-O- β -D-glucopyranoside (**3a**). Amorphous powder. $[\alpha]_{\text{D}}^{25} = 53.2^\circ$ (MeOH, c 0.4). ^1H NMR $[(\text{CD}_3)_2\text{CO} + \text{D}_2\text{O}]$: δ 3.35–3.55 (4H, *m*, glc-H-2, 3, 4, 5), 3.65 (1H, *dd*, $J = 11.1$ and 4.4 Hz, glc-H-6), 3.78 (1H, *dd*, $J = 2.6$ and 11.1 Hz, glc-H-6), 4.85 (1H, *d*, $J = 7.4$ Hz, glc-H-1), 6.83 (1H, *d*, $J = 8.1$ Hz, H-5), 7.51 (1H, *dd*, $J = 8.1$ and 1.8 Hz, H-6), 7.67 (1H, *d*, $J = 1.8$ Hz, H-2). ^{13}C NMR $[(\text{CD}_3)_2\text{CO} + \text{D}_2\text{O}]$: δ 61.8 (glc-C-6), 70.5 (glc-C-4), 74.1 (glc-C-2), 76.7 (glc-C-5), 77.5 (glc-C-3), 102.9 (glc-C-1), 116.8 (C-5), 119.1 (C-2), 123.2 (C-1), 126.9 (C-6), 145.9 (C-3), 152.4 (C-4), 169.4 (CO). High-resolution negative SIMS m/z (rel. int.): 315.0175 ($\text{C}_{13}\text{H}_{15}\text{O}_9$) $[\text{M} - \text{H}]^-$ (100).

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