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

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Key Word Index—*Lobelia sessilifolia*; Campanulaceae; hairy root culture; (+)- catechin; (−)-epicatechin; protocatechuic acid; gallic acid.

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.

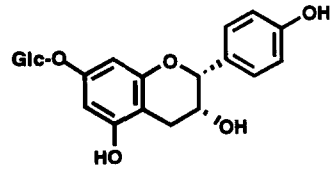
INTRODUCTION

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.

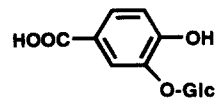
RESULTS AND DISCUSSION

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 (δ6.72 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.



2b



3a

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

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(7.4 Hz) of the glucose C-1 proton signal in the ^{1}H NMR spectrum. Therefore, **3a** was characterized as protocatechuic 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

^{1}H and ^{13}C NMR were measured at 270 and 67.5 MHz, respectively, locked to the major deuterium resonance of the solvent ($(CD_3)_2CO$). 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 × 30.5 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. $[\alpha]_D^{25}$ − 102.0° (MeOH, *c* 0.2). ^{1}H NMR [$(CD_3)_2CO$ + D_2O]: *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 [$(CD_3)_2CO$ + D_2O]: *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 $[M + H]^+$ (57).

Protocatechuic acid 3-O-β-D-*glucopyranoside* (**3a**). Amorphous powder. $[\alpha]_D^{25}$ − 53.2° (MeOH, *c* 0.4). ^{1}H NMR [$(CD_3)_2CO$ + D_2O]: *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 [$(CD_3)_2CO$ + D_2O]: *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 ($C_{13}H_{15}O_9$) $[M - H]^-$ (100).

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