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BIOTRANSFORMATION OF DIGITOXIGENIN BY CULTURED GINSENG CELLS

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Key Word Index—*Panax ginseng*; Araliaceae; cultured cells; biotransformation; digitoxigenin; malonyl conjugate; glucosylation; hydroxylation; acovenosigenin-A; digitoxigenin β-D-glucoside malonyl ester.

Abstract—Nine compounds, including a new compound (digitoxigenin β-D-glucoside malonyl ester), were isolated as biotransformation products of digitoxigenin by cell suspension cultures of *Panax ginseng* (Pg-3 cell line). At the same time, two known products were identified by TLC and HPLC.

INTRODUCTION

In order to obtain new and more effective cardenolides, we have investigated the biotransformation of digitoxigenin (**1**), a precursor of cardiac glycosides, by cultured *Digitalis* [1] and *Strophanthus* cells [2–5], the parent plants of which contain cardiac glycosides. Five new compounds, three esters and two glycosides, were obtained as crystals following the biotransformation compound **1**, ginseng hairy root cultures [6], the parent plant of which contains no cardiac glycosides. In the present paper, we report on the biotransformation of compound **1** by cultured ginseng cells.

RESULTS AND DISCUSSION

After incubation of digitoxigenin (**1**) (107) mg) with calli (1230 g fr. wt) for 18–20 days, digitoxigenin stearate (**2**), digitoxigenin palmitate (**3**), digitoxigenin myristate (**4**), digitoxigenin laurate (**5**), 3-epidigitoxigenin (**6**) (the main product in this experiment), periplogenin (**7**), 3-epiperiplogenin (**8**), 3-epidigitoxigenin β-D-glucoside (**9**) and digitoxigenin β-D-glucoside (**10**) were isolated as the biotransformation products in the yields shown in Table 1. Because of the small amounts of samples, periplogenin β-D-glucoside (**11**) and digitoxigenin β-D-sophoroside (**12**) were

Table 1. Biotransformation products of digitoxigenin (**1**) by ginseng cultured cells

Products		Yield, mg (%)	
		Cells	Medium
Digitoxigenin stearate	(**2**)	28.9 (1.6)	
Digitoxigenin palmitate	(**3**)	65.5 (3.7)	
Digitoxigenin myristate	(**4**)	29.0 (1.7)	
Digitoxigenin laurate	(**5**)	16.0 (1.0)	
3-Epidigitoxigenin	(**6**)	120.0 (11.2)	72.0 (6.7
Periplogenin	(**7**)	20.7 (1.9)	64.3 (5.8
3-Epiperiplogenin	(**8**)	*	18.8 (1.7
3-Epidigitoxigenin β-D-glucoside	(**9**)	12.0 (0.8)	
Digitoxigenin β-D-glucoside	(**10**)	111.5 (7.3)	*
Periplogenin β-D-glucoside	(**11**)	*	
Digitoxigenin β-D-sorphoroside	(**12**)	*	
Acovenosigenin-A	(**13**)†	*	8.0 (0.7
Digitoxigenin β-D-glucoside malonyl ester	(**14**)‡	42.9 (2.4)	*

*Identified with authentic samples.
‡New compound.
†New biotransformation product.

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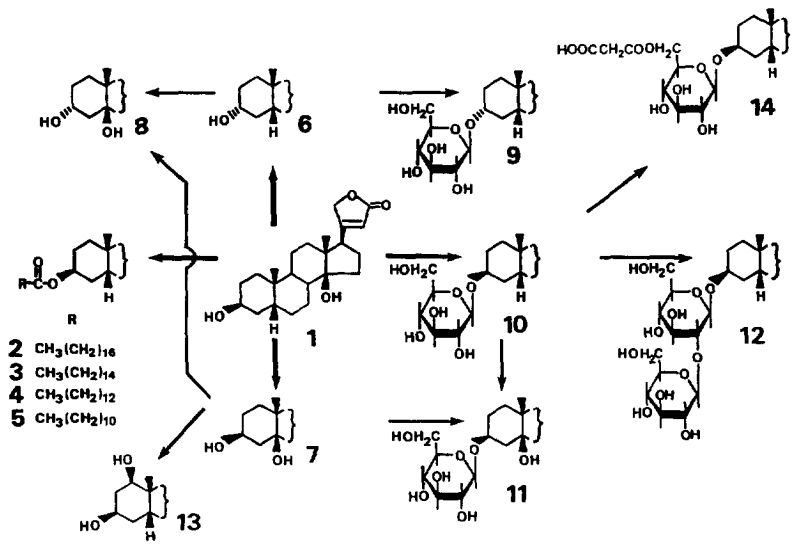

identified by comparison with authentic samples [6] by TLC and HPLC.

Acovenosigenin-A (1β-hydroxydigitoxitenin) (**13**) was isolated and identified with an authentic sample by TLC and HPLC as a new biotransformation product; 17βH-acovenosigenin-A has already been obtained in the studies using cultured *Strophanthus gratus* cells [2].

The FAB-mass spectrum of compound **14** exhibited a peak due to $[M+H]^+$ at *m/z* 623. In the ^1H NMR spectrum of compound **14**, the C-6′ proton signals of the glucopyranosyl moiety were shifted downfield to δ 4.75 (1H, *dd*, $J = 11.5$, 4.5 Hz) and 4.99 (1H, *br d*, $J = 11.5$ Hz) in comparison with C-6′ proton signals of compound **10**, and an anomeric proton signal was observed at δ 4.77 (1H, *d*, $J = 8$ Hz). In the ^1H and ^{13}C NMR spectra of **14**, the methylene proton signals were observed at δ 3.66 and 3.70 (each 1H, AB*q*, $J =$ 15.5 Hz) and the carbon signals were detected at δ 42.5, 167.8 and 169.3, suggesting that the acyl moiety was malonic acid. On the basis of the above spectral data, the structure of **14** was elucidated as the new compound, digitoxigenin-3β-*O*-(6′-*O*-malonyl-β-D-glucopyranoside) (digitoxigenin β-D-glucoside malonyl ester).

Because steryl glycoside fatty acid esters [7] and malonyl ginsenosides [8] are normal constituents of gingseng roots, it was recognized that some of the reactions (esterification and glycosylation) involved in their formation were utilized in the biotransformation of compound **1**, a substance foreign to ginseng. In the same way 18β-glycyrrhetinic acid was converted to its malonylated and glycosylated products in ginseng hairy root cultures [9].

EXPERIMENTAL

^1H and ^{13}C NMR: Varian XL-400 spectrometer; positive FAB-MS: Jeol JMS-DX300 instrument.

Culture methods. The cultured cells (Pg-3 cell line) of ginseng (*Panax ginseng* C. A. Meyer) used in this investigation were derived from seedling of this plant in 1983 and maintained on B2K agar medium (Murashige and Skoog's medium supplemented with sucrose (30 g l^{-1}), agar (9 g l^{-1}), indole-3-butyric acid (2 mg l^{-1}) and kinetin (0.1 mg l^{-1}) at 25° in the dark, as previously reported [10]. In the biotransformation experiments, 30 or 40 mg digitoxigenin (**1**) suspended in Tween 80 (5%) was added to each flask (250 ml B2K liquid medium per 1 l flask) followed by the calli (*ca* 30–40 g fresh wt per flask) from 4-week-old static cultures. The cultures were then incubated at 25° in the dark in a shaker (90 strokes min^{-1}) for 18–20 days.

Detection and separation of biotransformation products. After incubation of ditoxigenin (**1**) (1070 mg: 30 mg × 17 and 40 mg × 14) with the calli (1230 g fresh wt), the $CHCl_3$ and the $CHCl_3$–iso-PrOH (3:2) extracts from the calli and the medium, respectively, were obtained. The $CHCl_3$ extracts were examined by TLC. On spraying the plates with Kedde's reagent and 10% H_2SO_4, five Kedde-positive spots (R_f 0.64, 0.42, 0.30, 0.10, 0.06; $CHCl_3$–EtOH, 10:1) in addition to the one due to digitoxigenin (**1**) (R_f 0.45) were detected. Five Kedde-positive spots (R_f 0.54, 0.45, 0.41, 0.30, 0.19; $CHCl_3$–MeOH–H_2O 15:7:1) were also detected in the $CHCl_3$–*iso*-PrOH (3:2) extracts. The $CHCl_3$ extracts of the calli (6.5 g) and the medium (5.4 g) were chromatographed on a silica gel column (Wako gel C-200 and C-300, respectively). The $CHCl_3$–*iso*-PrOH (3:2) extracts from the calli and the medium were combined (2.9 g), and chromatographed on a silica gel column.

Isolation and identification of biotransformation products. Further purification of the products was achieved by rechromatography on a silica gel column using $CHCl_3$–MeOH solvent system and repeated HPLC (Senshu Pak. ODS-4301-N (300 × 10 mm), solvent 60% MeOH in H_2O or 55% MeOH in 0.05%

trifluoroacetic acid (TFA), flow rate: 3 ml min^{-1}), coupled to a UV detector and a differential refractometer. Products **2**–**10**, **13** and **14** were isolated in the yields shown in Table 1 and identified with authentic samples by TLC, HPLC and ^1H and ^{13}C NMR. Products **11** and **12** were identified with authentic samples by TLC and HPLC [2–6].

Digitoxigenin β-D-glucoside malonyl ester (**14**). Compound **14** was isolated after purification (especially, the fraction containing **14** was concentrated under 35°) by HPLC (R_t 24.6 min: solvent 55% MeOH in 0.05% TFA; flow rate: 3 ml min^{-1}). Amorphous solid, FAB-MS (low resolution) *m/z* (rel. int.): 623 $[M + H]^+$ (12), 605 $[M + H - H_2O]^+$ (9), 537 [M − malonic acid]$^+$ (7), 357 [aglycone + 2H − H_2O]$^+$ (100); ^1H NMR (400 MHz, C_5D_5N): δ 0.69 (3H, *s*, H_3-19), 0.87 (3H, *s*, H_3-18), 2.67 (1H, *dd*, *J* = 9, 5.5 Hz, H-17α), 3.66 and 3.70 (each 1H, AB*q*, *J* = 15.5 Hz, –OOC–$C\underline{H}_2$–COOH), 3.93 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 4.09 (1H, *dd*, *J* = 9, 9 Hz, H-4′), 4.27 (1H, *m*, $W_{1/2}$ = 8 Hz, H-3), 4.75 (1H, *dd*, *J* = 11.5, 4.5 Hz, H-6′a), 4.77 (1H, *d*, *J* = 8 Hz, H-1′), 4.91 (1H, *dd*, *J* = 18, 1.5 Hz, H-21a), 4.99 (1H, *br d*, *J* = 11.5 Hz, H-6′b), 5.19 (1H, *dd*, *J* = 18, 1.5 Hz, H-21b), 6.01 (1H, *dd*, *J* = 1.5, 1.5 Hz, H-22); ^{13}C NMR (100 MHz, C_5D_5N): δ 30.5 (*t*, C-1), 27.0 (*t*, C-2), 74.9 (*t*, C-3), 30.5 (*t*, C-4), 35.7 (*d*, C-5), 26.8 (*t*, C-6), 21.8 (*t*, C-7), 41.7 (*d*, C-8), 35.3 (*d*, C-9), 36.6 (*s*, C-10), 21.3 (*t*, C-11), 39.7 (*t*, C-12), 49.9 (*s*, C-13), 84.5 (*s*, C-14), 33.0 (*t*, C-15), 27.1 (*t*, C-16), 51.3 (*d*, C-17), 16.0 (*q*, C-18), 23.6 (*q*, C-19), 174.5 (*s*, C-20), 73.5 (*t*, C-21), 117.4 (*d*, C-22), 175.8 (*s*, C-23), 103.2 (*d*, C-1′), 74.9 (*d*, C-2′), 78.3 (*d*, C-3′), 71.5 (*d*, C-4′), 75.0 (*d*, C-5′), 65.5 (*t*, C-6′), 167.8 (*s*, –OO$\underline{C}$–CH_2–COOH), 42.5 (*t*, –OOC–$\underline{C}H_2$–COOH), 169.3 (*s*, –OOC–CH_2–$\underline{C}$OOH).

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