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BIOTRANSFORMATION OF DIGITOXIGENIN BY CELL SUSPENSION CULTURES OF *STROPHANTHUS INTERMEDIUS**

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Key Word Index—*Strophanthus intermedius*, Apocynaceae, cell cultures, biotransformation, 5β- and 16β-hydroxylation, glucosylation, cardenolides, digitoxigenin, 3-epigitoxigenin

Abstract—Biotransformation products of digitoxigenin by cell suspension cultures of *Strophanthus intermedius* were isolated and their structures elucidated as 3-epidigitoxigenin, 3-*epi*-17βH-digitoxigenin, gitoxigenin, periplogenin, 3-epigitoxigenin, 3-epiperiplogenin and digitoxigenin β-D-glucoside, respectively. Furthermore, 3-*epi*-17βH-periplogenin, 3-epidigitoxigenin β-D-glucoside and digitoxigenone were identified by TLC and HPLC Biotransformation reactions of the digitoxigenin molecule by plant cell cultures are summarized and discussed comparatively

INTRODUCTION

In two earlier papers, we reported on the biotransformation of digitoxigenin (**1**) by *Strophanthus gratus* [1] and *S. amboensis* [2] In order to obtain new and more effective cardiac glycosides, we have carried out the biotransformation of cardenolides and their precursors by plant cell cultures; digitoxin by *Digitalis purpurea* [3], pregnenolone by *Nicotiana tabacum* and *Sophora angustifolia* [4], progesterone by *N tabacum*, *S angustifolia* [4] and *D purpurea* [5], 5β-pregnane-3,20-dione and 5β-pregnanolone by *D purpurea* [6] and digitoxigenin (**1**) by *D purpuea* [7]

Strophanthus intermedius (Apocynaceae) is a cardenolide-bearing plant [8, 9], however there has been no report on the use of tissue cultures of this plant until now. In this paper we wish to report the biotransformation of digitoxigenin (**1**) by a cell suspension culture derived from the stems of *S intermedius* and to discuss the biotransformation reactions of **1** by plant cell cultures

RESULTS AND DISCUSSION

The cell strain used for this work was derived from the stems of *Strophanthus intermedius*. However, no cardenolides were detected by TLC analysis of extracts of the cells, the same result has also been observed with *S. gratus* and *S amboensis* cells [1, 2] After digitoxigenin (**1**) (810.0 mg) was incubated with the cells (1.4 kg fr wt) for 18 days, the biotransformation products of **1** were extracted according to the method described in previous papers [1, 2]. Nine Kedde-positive spots in addition to the unchanged **1** were detected on TLC of the chloroform extracts and four Kedde-positive spots were found in the chloroform–methanol (2 1) extracts. After these extracts were combined (8.0 g) and separated, products **2**–**5**, **6**-diacetate, **7**,**7**-acetate and **9**-tetraacetate were isolated as crystalline compounds and their chemical structures were elucidated. Furthermore, products **8**, **10**-tetraacetate and **11** were identical with authentic samples by HPLC and TLC.

The unchanged **1** was recovered as colourless needles (35 0 mg). The biotransformation percentage yield was calculated from the ratio of the amount of the isolated product to the starting amount (775 0 mg) of **1**, taking into consideration their M_rs

Product **2** (68 0 mg; yield 8 8%), the main product in this experiment, had the composition $C_{23}H_{34}O_4$ on the basis of high-resolution mass spectrometry and its structure was determined as 3α,14-dihydroxy-5β,14β-card-20(22)-enolide (3-epidigitoxigenin) through the ^{1}H NMR spectral data (Experimental). Compound **2** has been identified previously as a product of the biotransformation of **1** in *S. amboensis* and *D. purpurea* cell cultures [2, 7]

Product **3** (6.0 mg; yield 0 8%) had the molecular formula $C_{23}H_{34}O_4$ (high-resolution mass spectrometry). In the ^{1}H NMR spectrum of **3**, the chemical shift values of H-17 and H_3-18 were shifted downfield to δ 3.18 (1H, *dd*, J=9 5, 9 5 Hz) and 1.03 (3H, *s*), and the H-3 signal was observed as a multiplet ($W_{1/2}$=24 Hz) at 3.56 From these spectral data the structure of **3** was established to be 3α,14-dihydroxy-5β,14β,17α-card-20(22)-enolide (3-*epi*-17βH-digitoxigenin) Isomerization of the 17β-lactone ring of **1**, which has been observed with *S. gratus* cell cultures [1], was also performed slightly with the *S. intermedius* cells.

Product **4**-diacetate (4.5 mg; yield 0 5%; $C_{27}H_{38}O_7$, high-resolution mass spectrometry) was isolated after acetylation and its structure elucidated as 3β,16β-diacetoxy-3β,14,16β-trihydroxy-5β,14β-card-20(22)-enolide (gitoxigenin diacetate) by comparing the ^{13}C NMR spectral data of **4**-diacetate with the values reported by Tori *et al* [10]. We could observe 16β-hydroxylation of digitoxin [3], but not of digitoxigenin (**1**) [7], by *D purpurea* However 16β-hydroxylation of **1** was now demonstrated in the cell cultures of *S. intermedius* (Table 1).

Product **5** (7.0 mg; yield 0.9%; $C_{23}H_{34}O_5$, high-resolution mass spectrometry) was identified as 3β,5,14-

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Table 1 Biotransformation reactions of the digitoxigenin (1) molecule by plant cell cultures

Plant species	Oxidation (3β-OH →3-Keto)	Epimerization (3β-OH →3α-OH)	Hydroxylation (1β-	4β-	5β-	12β-	16β-)	Glycosylation	Isomerization (17β- →17α-lactone ring)	Reference
Digitalis lanata	+					+		+ (digitoxoside)		[13, 15]
D purpurea	+	+			+			+		[7, 15]
Thevetia neriifolia	+							+		[14]
Daucus carota					+					[11]
Strophanthus gratus		+	+	+	+				+	[1]
S amboensis		+			+			+		[2]
S intermedius	+	+			+		+	+	+	

trihydroxy-5β,14β-card-20(22)-enolide (periplogenin) by comparison with the authentic compound (^{1}H and ^{13}C NMR) [2] 5β-Hydroxylation of **1** was observed with not only *D purpurea* [7] and *Daucus carota* [11] but also three species of *Strophanthus* cultured cells (Table 1)

Product **6**-diacetate (8 0 mg, yield 0 8%; $C_{27}H_{38}O_7$, high-resolution mass spectrometry) was isolated after acetylation. The ^{1}H NMR spectral data of **4**-diacetate and **6**-diacetate were similar to each other except for the proton signal of H-3, at δ 5 08 (1H, *br s*, $W_{1/2}$ = 7 Hz, H-3α) and 4 72 (1H, *m*, $W_{1/2}$ = 23 Hz, H-3β) In the ^{13}C NMR spectral data for C-1 to C-10 of **4**-diacetate and **6**-diacetate, moderate differences were also observed From these data, the structure of **6**-diacetate was established to be 3α,16β-diacetoxy-3α,14,16β-trihydroxy-5β, 14β-card-20(22)-enolide (3-epigitoxigenin diacetate) 3-Epigitoxigenin (**6**), which has been synthesized chemically [12], is a new biotransformation product produced by plant cell cultures

Product **7** (6 5 mg, yield 0 8%, $C_{23}H_{34}O_5$, high-resolution mass spectrometry) was isolated and the structure of **7** was identified as 3α,5,14-trihydroxy-5β,14β-card-20(22)-enolide (3-epiperiplogenin) by comparison with the authentic compound (^{1}H and ^{13}C NMR) [2] After acetylation 7-acetate (50 3 mg, yield 5.6%, $C_{25}H_{36}O_6$, high-resolution mass spectrometry) was obtained and its structure was confirmed as 3α-acetoxy-3α,5,14-trihydroxy-5β,14β-card-20(22)-enolide (3-epiperiplogenin acetate) through the mass spectral and ^{1}H and ^{13}C NMR spectral data. The formation of 3-epiperiplogein (**7**) was also demonstrated in the *S. amboensis* cells [2]

Product **8** was detected as the minor component by HPLC (R_t 6.9 min solvent 80% MeOH in H_2O) and identified with authentic 17βH-periplogenin [1] by HPLC and TLC (R_f 0 08, $CHCl_3$–EtOH, 10 1)

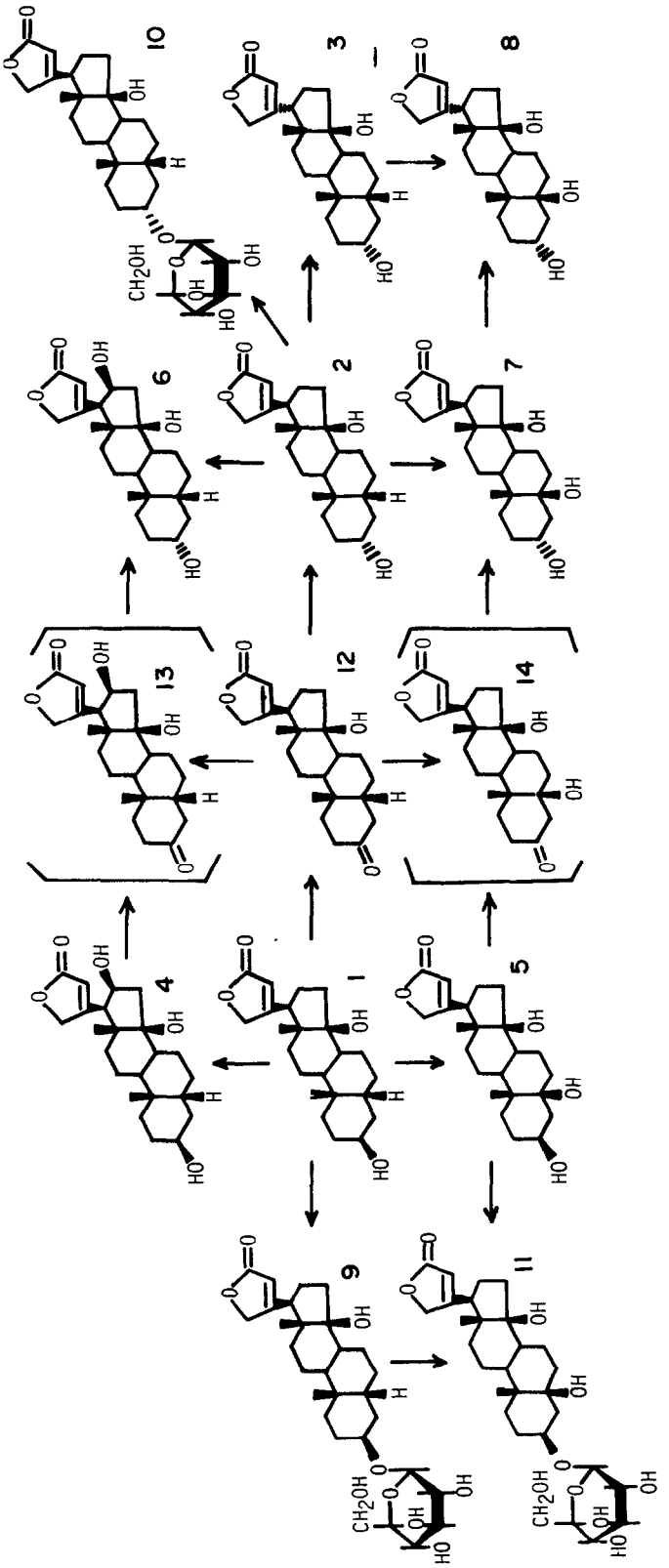
Product **9**-tetraacetate (5 0 mg, yield 0 3%) was isolated after acetylation, had molecular formula $C_{37}H_{52}O_{13}$ (high-resolution mass spectrometry) The structure of **9**-tetraacetate was determined as digitoxigenin β-D-glucoside tetraacetate by the mass spectral and ^{1}H NMR spectral data At the same time, **10**-tetraacetate in the acetylated fraction was identified with authentic 3-epidigitoxigenin β-D-glucoside tetraacetate by HPLC and TLC. Glycosylation of **1** containing digitoxoside formation had been demonstrated with *D lanata* [13], *D purpurea* [7], *Thevetia neriifolia* [14] and *S amboensis cell cultures* [2]. Furthermore, the formation of **9** and **10** had been performed by the cell cultures of *D purpurea*, with production of **10** predominating over **9** [7] On the other hand, the different extents of glucosylation, production of **9** predominating over **10** in spite of remaining **2**, was similarly observed in the *S amboensis* [2] and *S intermedius* cells

Product **11** was not isolated as a crystalline compound but instead identified with authentic periplogenin β-D-glucoside [2] by HPLC (R_t 9 8 min solvent 60% MeOH in H_2O) and TLC (R_f 0 14, CH_2Cl_2–MeOH–H_2O, 84 15 1)

After three days incubation of **1** (60 mg) with the *S intermedius* cells (228 g fr wt), the sole product **12** was detected on TLC (R_f 0 60, $CHCl_3$–EtOH, 10·1) and identified with authentic digitoxigenone [7] The 3β-hydroxyl of **1** was oxidized first to the 3-keto before the other reactions proceeded in the *S intermedius* cells. Oxidation of the 3β-hydroxyl of **1** had been also demonstrated with cell cultures of *D lanata* [13, 15], *D. purpurea* [7, 15] and *Thevetia neriifolia* [14]

The possible biotransformation pathway of **1** by cell suspension cultures of *S intermedius* is shown in Scheme 1 It was demonstrated that epimerization of the 3β-hydroxyl to the 3α-hydroxyl via 3-keto compounds such as **12**–**14** (**1** to **2**, **4** to **6** and **5** to **7**), 5β-hydroxylation (**1** to **5**, **2** to **7**, **3** to **8**, **9** to 11 and **12** to **14**), 16β-hydroxylation (**1** to **4**, **2** to **6** and **12** to **13**), glucosylation (**1** to **9**, **2** to **10** and **5** to **11**) and isomerization of the 17β-lactone ring (**2** to **3** and **7** to **8**) proceed in the *S intermedius* cells However, we could not detect compounds **13** and **14** in this experiment. The formation of 3-epigitoxigenin (**6**), which was newly obtained, was presumed to be produced by epimerization of gitoxigenin (**4**) or 16β-hydroxylation of 3-epidigitoxigenin (**2**)

The biotransformation reactions of the digitoxigenin (**1**) molecule by plant cell cultures are summarized in Table 1, together with the reactions described in the reviews [16, 17] It seems that oxidation and epimerization of the 3β-hydroxyl, 5β-hydroxylation and glucosylation are common reactions in plant cell cultures except for a few species On the other hand, 1β-, 4β-, 12β- and 16β-hydroxylation and isomerization of the 17β-lactone ring are probably specific abilities of plant cell cultures from the different origins It is to be expected that the biotransformation reactions with plant cell cultures, which were not previously known even in microbial transformation [18, 19], will contribute to provide new and more effective cardiac glycosides, some of which may be useful in the pharmaceutical industry [20]



Scheme 1 Biotransformation of digitoxigenin (1) by cell suspension cultures of *Strophanthus intermedius*

EXPERIMENTAL

Mps uncorr NMR 300 and 400 MHz ($CDCl_3$ or CD_3OD) HPLC of the biotransformation products was performed using a Nucleosil 5C18 (10 × 300 mm) column, coupled to a UV detector and a differential refractometer

Culture methods The stems of *Strophanthus intermedius* were sterilized by 70% EtOH and a saturated soln of bleaching powder and then rinsed with sterile H_2O and cut into *ca* 3 mm segments These segments were placed on modified Murashige and Skoog's tobacco medium containing 1 0 ppm 2,4-dichlorophenoxyacetic acid, 0 1 ppm kinetin and 3% sucrose in Jan 1983 The calli were subcultured at 30° in the dark every 4 weeks In the biotransformation experiments, the calli were transferred to a liquid medium containing digitoxigenin (**1**) suspended with Tween 80, and incubated in a shaker (90 spm) for 3 or 18 days

Detection and separation of biotransformation products Digitoxigenin (**1**) (810 0 mg) was added to the calli (1 4 kg fr wt) from 4-week-old static cultures, and after 18 days, the $CHCl_3$ and the $CHCl_3$–MeOH (2 1) extracts from the calli and the medium were obtained according to the method described in a previous paper [1] The $CHCl_3$ extracts from the calli and the medium were examined on TLC with Kedde's reagent and 10% H_2SO_4, and nine Kedde-positive spots (R_f 0 41, 0 37, 0 29, 0 26, 0 21, 0 17, 0 10, 0 04, 0 01, $CHCl_3$–EtOH, 10 1) except digitoxigenin (**1**) (R_f 0 45, $CHCl_3$–EtOH, 10 1) were detected Four Kedde-positive spots (R_f 0 34, 0 29, 0 24, 0 14, CH_2Cl_2–MeOH–H_2O, 84 15 1) were detected similarly in the $CHCl_3$–MeOH (2 1) extracts These extracts were combined (8 0 g), chromatographed on a silica gel column (350 g Wako gel C-200) and eluted as follows fraction A, $CHCl_3$ (3 5 l), fraction B, $CHCl_3$–MeOH (97 3, 5 0 l), fraction C, $CHCl_3$–MeOH (93 7, 3 0 l), fraction D, $CHCl_3$–MeOH (17 3, 1 0 l) and fraction E, $CHCl_3$–MeOH (7 3, 1 0 l)

Recovery of digitoxigenin (**1**) Fraction A yielded the unchanged **1**, and crude products **2** and **3** (R_f 0 45, 0 41, 0 37, $CHCl_3$–EtOH, 10 1) Further purification of these compounds was achieved by repeated HPLC (R_t 15 4 min, 14 8 min and 14 0 min Nucleosil 5C18, 70% MeOH in H_2O, flow rate 3 ml/min) **1** (R_t 14 8 min) was recrystallized from EtOH–H_2O to give colourless needles (35 mg) and identified with authentic digitoxigenin (**1**) by HPLC and TLC

Isolation of 3-epidigitoxigenin (**2**) Product **2** (68 0 mg) was isolated from the fraction containing the peak at 15 4 min **2**, mp 268–270° (from MeOH), $C_{23}H_{34}O_4$ (required 374 2457, $[M]^+$ at *m/z* 374 2468), IR ν_{max}^{KBr} cm^{-1} 3430, 1745, 1635 ^1H NMR (400 MHz, $CDCl_3$) δ 0 80 (3H, *s*, Me-18), 0 85 (3H, *s*, Me-19), 2 71 (1H, *dd*, J = 9, 6 Hz, H-17 α), 3 59 (1H, *m*, $W_{1/2}$ = 26 Hz, H-3β), 4 53 (1H, *dd*, J = 18, 2 Hz, H-21a), 4 92 (1H, *dd*, J = 18, 2 Hz, H-21b), 5 80 (1H, *dd*, J = 2, 2 Hz, H-22) EIMS *m/z* (rel int) 374 $[M]^+$ (6), 356 $[M-H_2O]^+$ (25), 338 $[M-2\times H_2O]^+$ (4), 246 $[C_{17}H_{26}O]^+$ (34), 203 $[C_{15}H_{23}]^+$ (100), 162 $[C_{12}H_{18}]^+$ (20), 147 $[C_{11}H_{15}]^+$ (14)

Isolation of 3-epi-17βH-*digitoxigenin* (**3**) Product **3** (6 0 mg) was isolated from the fraction containing the peak at 14 0 min by the same methods described above for **2** Compound **3**, mp 161–163° (from MeOH–H_2O), $C_{23}H_{34}O_4$ (required 374 2457, $[M]^+$ at *m/z* 374 2453), IR ν_{max}^{KBr} cm^{-1} 3400, 1780 (sh), 1720, 1615 ^1H NMR (400 MHz, CD_3OD) δ 0 95 (3H, *s*, Me-19), 1 03 (3H, *s*, Me-18), 3 18 (1H, *dd*, J = 9 5, 9 5 Hz, H-17β), 3 56 (1H, *m*, $W_{1/2}$ = 24 Hz, H-3β), 4 85 (1H, *ddd*, J = 18, 2, 1 Hz, H-21a), 4 95 (1H, *dd*, J = 18, 2 Hz, H-21b), 5 95 (1H, *dd*, J = 2, 2 Hz, H-22) EIMS *m/z* (rel int) 374 $[M]^+$ (5), 356 $[M-H_2O]^+$ (21), 246 $[C_{17}H_{26}O]^+$ (33), 203 $[C_{15}H_{23}]^+$ (100), 189 $[C_{14}H_{21}]^+$ (20), 162 $[C_{12}H_{18}]^+$ (21)

Isolation of gitoxigenin (**4**) *diacetate* Fraction B yielded the crude product **4** After acetylation with pyridine–Ac_2O at room temp and purification by HPLC (R_t 7 6 min solvent 90% MeOH in H_2O), compound **4**-diacetate was recrystallized from MeOH (4 5 mg), mp 250–253°, $C_{27}H_{38}O_7$ (required 474 2618, $[M]^+$ at *m/z* 474 2621) ^1H NMR (300 MHz, $CDCl_3$) δ 0 93 (3H, *s*, Me-18), 0 95 (3H, *s*, Me-19), 1 78 (1H, *dd*, J = 16, 2 5 Hz, H-15α), 1 96 (3H, *s*, AcO-16β), 2 05 (3H, *s*, AcO-3β), 2 73 (1H, *dd*, J = 16, 9 Hz, H-15β), 3 19 (1H, *d*, J = 9 Hz, H-17α), 4 84 (1H, *dd*, J = 18, 2 Hz, H-21a), 4 98 (1H, *dd*, J = 18, 2 Hz, H-21b), 5 08 (1H, *br s*, $W_{1/2}$ = 7 Hz, H-3α), 5 48 (1H, *ddd*, J = 9, 9, 2 5 Hz, H-16α), 5 97 (1H, *dd*, J = 2, 2 Hz, H-22) ^{13}C NMR (75 MHz, $CDCl_3$) δ 15 9 (*q*), 20 8 (*t*), 20 9 (*t*), 21 0 (*q*), 21 4 (*q*), 23 6 (*q*), 25 0 (*t*), 26 1 (*t*), 30 4 (*t*, *t*), 35 0 (*s*), 35 6 (*d*), 36 7 (*d*), 39 1 (*t*), 41 2 (*t*), 41 7 (*d*), 50 0 (*s*), 56 1 (*d*), 70 2 (*d*), 73 9 (*d*), 75 6 (*t*), 84 2 (*s*), 121 4 (*d*), 167 7 (*s*), 170 4 (*s*), 170 6 (*s*), 174 0 (*s*) EIMS *m/z* (rel int) 474 $[M]^+$ (1), 432 $[M-C_2H_2O]^+$ (6), 414 $[M-HOAc]^+$ (14), 354 $[M-2\times HOAc]^+$ (24), 336 $[M-2\times HOAc-H_2O]^+$ (8), 204 $[C_{15}H_{24}]^+$ (27), 203 $[C_{15}H_{23}]^+$ (100)

Isolation of periplogenin (**5**) Product **5** (7 0 mg) was isolated from fraction B after purification by HPLC (R_t 8 1 min solvent 80% MeOH in H_2O) Compound **5**, mp 136–140 (from MeOH), $C_{23}H_{34}O_5$ (required 390 2405, $[M]^+$ at *m/z* 390 2398), IR ν_{max}^{KBr} cm^{-1} 3320, 1775, 1740, 1620 ^1H NMR (400 MHz, CD_3OD) δ 0 88 (3H, *s*, Me-18), 0 93 (3H, *s*, Me-19), 2 84 (1H, *dd*, J = 9, 6 Hz, H-17α), 4 12 (1H, *br s*, $W_{1/2}$ = 7 Hz, H-3α), 4 91 (1H, *dd*, J = 18, 2 Hz, H-21a), 5 03 (1H, *dd*, J = 18, 2 Hz, H-21b), 5 89 (1H, *dd*, J = 2, 2 Hz, H-22) EIMS *m/z* (rel int) 390 $[M]^+$ (1), 372 $[M-H_2O]^+$ (16), 354 $[M-2\times H_2O]^+$ (22), 318 $[C_{19}H_{26}O_4]^+$ (100), 300 $[C_{19}H_{24}O_3]^+$ (7), 262 $[C_{17}H_{26}O_2]^+$ (9), 219 $[C_{15}H_{23}O]^+$ (40), 201 $[C_{15}H_{21}]^+$ (59), 145 $[C_{11}H_{13}]^+$ (18)

Isolation of 3-*epigitoxigenin* (**6**) *diacetate* The crude product **6** obtained from fraction C was purified by HPLC(R_t 8 4 min solvent 90% MeOH in H_2O) after acetylation with pyridine–Ac_2O at room temp **6**-Diacetate (8 0 mg), mp 119–125 (from MeOH–H_2O), $C_{27}H_{38}O_7$ (required 474 2617, $[M]^+$ at *m/z* 474 2608). ^1H NMR (400 MHz, $CDCl_3$). δ 0 92 (3H, *s*, Me-18), 0 93 (3H, *s*, Me-19), 1 79 (1H, *dd*, J = 16, 2 5 Hz, H-15α), 1 96 (3H, *s*, AcO-16β), 2 04 (3H, *s* AcO-3α), 2 78 (1H, *dd*, J = 16, 9 Hz, H-15β), 3 20 (1H, *d*, J = 9 Hz, H-17α), 4 72 (1H, *m*, $W_{1/2}$ = 23 Hz, H-3β), 4 85 (1H, *dd*, J = 18, 2 Hz, H-21a), 4 99 (1H, *dd*, J = 18, 2 Hz, H-21b), 5 49 (1H, *ddd*, J = 9, 9, 2 5 Hz, H-16α), 5 97 (1H, *dd*, J = 2, 2 Hz, H-22) ^{13} NMR (100 MHz, $CDCl_3$) δ 15 9 (*q*), 20 6 (*t*), 21 0 (*t*, *q*), 21 4 (*q*), 23 1 (*q*), 26 6 (*t*), 26 7 (*t*), 32 1 (*t*), 34 6 (*s*), 34 7 (*t*), 36 1 (*d*), 39 1 (*t*), 41 2 (*d*, *t*), 41 8 (*d*), 49 9 (*s*), 56 0 (*d*), 73 9 (*d*, *d*), 75 6 (*t*), 84 2 (*s*), 121 4 (*d*), 167 7 (*s*), 170 4 (*s*), 170 7 (*s*), 174 1 (*s*). EIMS *m/z* (rel int) 474 $[M]^+$ (1), 414 $[M-HOAc]^+$ (12), 354 $[M-2\times HOAc]^+$ (17), 336 $[M-2\times HOAc-H_2O]^+$ (7), 204 $[C_{15}H_{24}]^+$ (21), 203 $[C_{15}H_{23}]^+$ (100), 178 (20)

Isolation of 3-*epiperiplogenin* (**7**) From a part of fraction C, product **7** (6 5 mg) was isolated after purification by HPLC (R_t 7 2 min solvent 80% MeOH in H_2O) **7**, mp 232–235° (from MeOH–H_2O), $C_{23}H_{34}O_5$ (required 390 2405, $[M]^+$ at *m/z* 390 2383), IR ν_{max}^{KBr} cm^{-1} 3400, 1750, 1630 ^1H NMR (300 MHz, $CDCl_3$) δ 0 88 (3H, *s*, Me-18), 0 89 (3H, *s*, Me-19), 2 79 (1H, *dd*, J = 9, 6 Hz, H-17α), 4 05 (1H, *m*, $W_{1/2}$ = 22 Hz, H-3β), 4 81 (1H, *dd*, J = 18, 2 Hz, H-21a), 4 99 (1H, *dd*, J = 18, 2 Hz, H-21b), 5 89 (1H, *dd*, J = 2, 2 Hz, H-22) EIMS *m/z* (rel int) 390 $[M]^+$ (4), 372 $[M-H_2O]^+$ (19), 354 $[M-2\times H_2O]^+$ (20), 336 $[M-3\times H_2O]^+$ (4), 318 $[C_{19}H_{26}O_4]^+$ (4), 262 $[C_{17}H_{26}O_2]^+$ (7), 247 $[C_{16}H_{23}O_2]^+$ (10), 219 $[C_{15}H_{23}O]^+$ (18), 201 $[C_{15}H_{21}]^+$ (100), 160 $[C_{12}H_{16}]^+$ (12), 145 $[C_{11}H_{13}]^+$ (12) From most part of fraction C, **7**-acetate (50 3 mg) was isolated after acetylation and purification by rechromatography on silica gel (Wako gel C-300) **7**-Acetate (R_f 0 16, C_6H_6–Me_2CO, 3 1), mp 225–229° (from MeOH–H_2O), $C_{25}H_{36}O_6$ (required 432 2512, $[M]^+$ at *m/z* 432 2528), IR ν_{max}^{KBr} cm^{-1} 3430, 1730, 1720, 1630, 1245 ^1H NMR

(300 MHz, $CDCl_3$) δ 0.91 (3H, *s*, Me-18), 0.93 (3H, *s*, Me-19), 2.05 (3H, *s*, AcO-3α), 2.81 (1H, *dd*, J = 9, 6 Hz, H-17α), 4.82 (1H, *dd*, J = 18, 2 Hz, H-21a), 5.01 (1H, *dd*, J = 18, 2 Hz, H-21b), 5.11 (1H, *m*, $W_{1/2}$ = 22 Hz, H-3β), 5.91 (1H, *dd*, J = 2, 2 Hz, H-22) EIMS *m/z* (rel int.) 432 $[M]^+$ (4), 372 $[M-HOAc]^+$ (20), 354 $[M-HOAc-H_2O]^+$ (26), 336 $[M-HOAc-2\times H_2O]^+$ (4), 318 (4), 247 (13), 219 (16), 201 (100), 160 (11), 145 (11)

Identification of 3-epi-17βH-*periplogenin* (**8**). When **7** was purified by HPLC, the minor component contained in the peak at 6.9 min was detected. The product was not isolated but identified with authentic 3-*epi*-17βH-periplogenin [1] by TLC (R_f 0.08, $CHCl_3$–EtOH, 10 1) and HPLC

Isolation of digitoxigenin β-D-*glucoside* (**9**) *tetraacetate* After acetylation of fraction D and purification by HPLC (R_t 26.3 min. solvent 70% MeOH in H_2O), product **9**-tetraacetate was recrystallized from MeOH–H_2O (5.0 mg), mp 168–170°, $C_{37}H_{52}O_{13}$ (required 704.3407, $[M]^+$ at *m/z* 704.3411). ^{1}H NMR (400 MHz, $CDCl_3$). δ 0.87 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-19), 2.01 (3H, *s*, AcO–), 2.02 (6H, *s*, AcO– × 2), 2.08 (3H, *s*, AcO–), 2.77 (1H, *dd*, J = 9, 5.5 Hz, H-17α), 3.67 (1H, *ddd*, J = 9.5, 5, 2.5 Hz, H-5'), 4.01 (1H, *br s*, $W_{1/2}$ = 7.5 Hz, H-3α), 4.12 (1H, *dd*, J = 12, 2.5 Hz, H-6'), 4.25 (1H, *dd*, J = 12, 5 Hz, H-6''), 4.55 (1H, *d*, J = 8 Hz, H-1'), 4.80 (1H, *dd*, J = 18, 1.8 Hz, H-21a), 4.98 (1H, *dd*, J = 18, 1.8 Hz, H-21b), 5.00 (1H, *dd*, J = 9.5, 8 Hz, H-2'), 5.08 (1H, *dd*, J = 9.5, 9.5 Hz, H-4'), 5.22 (1H, *dd*, J = 9.5, 9.5 Hz, H-3'), 5.88 (1H, *dd*, J = 1.8, 1.8 Hz, H-22). EIMS *m/z* (rel int.) 704 $[M]^+$ (2), 357 $[C_{23}H_{33}O_3]^+$ (100), 331 $[C_{14}H_{19}O_9]^+$ (66), 246 $[C_{17}H_{26}O]^+$ (27), 203 $[C_{15}H_{23}]^+$ (50), 169 (61)

Identification of 3-*epidigitoxigenin* β-D-*glucoside* (**10**) *tetraacetate*. During **9**-tetraacetate purification by HPLC, the minor component, a peak at 31.5 min was detected. Because of the small amount of sample, the product was not isolated but instead identified with authentic 3-epidigitoxigenin β-D-glucoside tetraacetate [8] by HPLC and TLC, R_f 0.56: the first development with $CHCl_3$–EtOH (7 1), the second development with C_6H_6–Me_2CO (3 1)

Identification of periplogenin β-D-*glucoside* (**11**). Because of the small amount of sample, product **11** (from fraction E) was not isolated as a crystalline compound but instead identified with authentic periplogenin β-D-glucoside [2] by HPLC (R_t 9.8 min. solvent 60% MeOH in H_2O) and TLC (R_f 0.14, CH_2Cl_2–MeOH–H_2O, 84 15.1)

Identification of digitoxigenone (**12**) After **1** (60 mg) was incubated for 3-days with the calli (228 g fr wt), sole Kedde-positive spot (R_f 0.60; $CHCl_3$–EtOH, 10·1) except **1** was detected in the $CHCl_3$ extracts from the calli and the medium. The product was identified with authentic digitoxigenone [7].

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