Pregnane and Pregnane Glycosides from *Trachelospermum liukiuense*[1)]

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Cortexone and three new bisdesmosidic glycosides of teikagenin were isolated from *Trachelospermum liukiuense*, together with five glycosides already known in *Trachelospermum asiaticum*. The new glycosides have D-digitalose linked to the 20-OH as well as to the 3-OH.

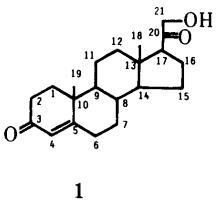
Keywords *Trachelospermum*; Apocynaceae; pregnane; cortexone; teikagenin; 20*S*,5α-pregn-6-en-3β,17α,20-triol; teikagenin 3,20-bis-*O*-digitaloside; teikaside

In the course of studies on the constituents of genus *Trachelospermum*, we have investigated the pregnane glycosides,[1-3)] lignans[4)] and triterpenoids[5,6)] from *Trachelospermum asiaticum* NAKAI. The bisdesmosidic glycosides of 20*S*,5α-pregn-6-en-3β,17α,20-triol (teikagenin) with a β-D-digitalosyl or 4-*O*-acetyl-L-sarmentosyl-(1→4)-β-D-digitalosyl group at the 3-OH and 2,6-dideoxy-3-*O*-methylhexoses at the 20-OH (teikasides) were isolated and their structures were established.

The species indigenous to the Ryukyu Islands is classified as *Trachelospermum liukiuense* HATSUSIMA. In order to compare the constituents of the two species, the pregnane glycosides from *T. liukiuense* were investigated. This paper deals with the isolation and structure identification of cortexone and bisdesmosidic glycosides of teikagenin.

When the MeOH percolate was partitioned with benzene, $CHCl_3$ and then BuOH in the usual manner, compound **1**, showing less polar behavior on thin layer chromatography (TLC), was isolated from the benzene extract. Based on the M^+ peak of **1** at *m/z* 330.220 ($C_{21}H_{30}O_3$) in the electron impact (EI) mass spectrum (MS) and that of **1**-monoacetate (**1a**) at *m/z* 372.227 ($C_{23}H_{32}O_4$), **1** seemed to be a pregnane. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, methylene protons in a primary carbinol group (δ 4.45 and 4.53, doublet each, J=19 Hz) and one olefinic proton (δ 5.86) were observed together with two tertiary methyl groups, and **1** was considered to be cortexone (deoxycorticosterone). The structure of **1** was confirmed by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral comparisons with authentic cortexone and cortexone acetate, as well as by TLC and high-performance liquid chromatography (HPLC).

From the $CHCl_3$ extract, three new compounds (**2**—**4**) were isolated together with the known teikagenin bisdesmosidic glycosides (**5**—**9**), by chromatographies on a polystyrene (MCI gel) column, an octadecyl silica (ODS)



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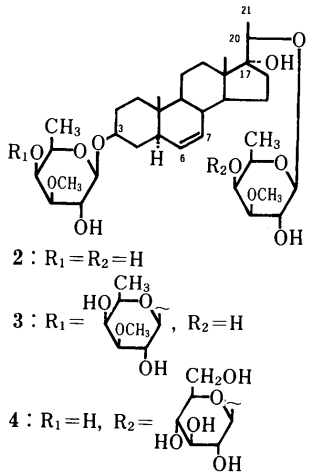

2 : R_1=R_2=H

3 : R_1= , R_2=H

4 : R_1=H, R_2=

Chart 1

TABLE I. ^{1}H Chemical Shifts of Compounds **2**—**4**, δ (ppm) from TMS in Pyridine-d_5 (J/Hz in Parentheses)

	2	**3**		**4**	
H-3α	3.98 (m)	3.93[c)] (m)		3.95[e)] (m)	
H-6	5.54 (br d, 10)	5.53 (br d, 10)		5.55 (br d, 10)	
H-7	5.33 (br d, 10)	5.32 (br d, 10)		5.33 (br d, 10)	
H-18, 19	0.75, 0.78	0.72, 0.77		0.75, 0.76	
H-20	4.17 (q, 6)	4.17 (q, 6)		4.11 (q, 6)	
H-21	1.63 (d, 6)	1.63 (d, 6)		1.61 (d, 6)	
3-*O*-Sugars	(digit.)[a)]	(digit.-1)	(digit.-2)	(digit.)	
H-1	4.84 (d, 8)	4.76[c)] (d, 8)	4.86[d)] (d, 8)	4.82[e)] (d, 8)	
H-2	4.34 (dd, 8, 9)	4.34 (dd, 8, 9)	4.32 (dd, 8, 9)	4.35 (dd, 8, 9)	
H-3	3.53 (dd, 9, 3)	3.61 (dd, 9, 3)	3.46 (dd, 9, 3)	3.53 (dd, 9, 3)	
H-4	4.08 (d, 3)	4.21[d)] (d, 3)	4.01 (d, 3)	4.08[f)] (d, 3)	
H-5	3.82 (q, 6)	3.75—3.85		3.81 (q, 6)	
H-6	1.59 (d, 6)	1.56[b)] (d, 6)	1.60[b)] (d, 6)	1.59 (d, 6)	
3-*O*-Me	3.60	3.73	3.57	3.60[f)]	
20-*O*-Sugars	(digit.)[a)]	(digit.)		(digit.)	(glc.)
H-1	4.82 (d, 8)	4.85 (d, 8)		4.78 (d, 8)	5.13[g)] (d, 8)
H-2	4.31 (dd, 8, 9)	4.32 (dd, 8, 9)		4.38 (dd, 8, 9)	3.95 (dd, 8, 9)
H-3	3.50 (dd, 9, 3)	3.50 (dd, 9, 3)		3.55 (dd, 9, 3)	4.21 (t, 9)
H-4	4.07 (d, 3)	4.07 (d, 3)		4.32[g)] (d, 3)	4.16 (t, 9)
H-5	3.80 (q, 6)	3.75—3.85		3.77 (q, 6)	3.95 (m)
H-6	1.58 (d, 6)	1.58 (d, 6)		1.58 (d, 6)	4.55 (dd, 12, 2) 4.34 (dd, 12, 4)
3-*O*-Me	3.56	3.56		3.65	

a, *b*) Signal assignments marked *a*) or *b*) may be reversed. *c*—*g*) NOE was observed between the signals marked *c*—*f*) or *g*).

TABLE II. ^{13}C Chemical Shifts of Compounds **1**—**4**, δ (ppm) from TMS in Pyridine-d_5

	1	2	3[a]		4[a]	
C-1	34.3[b]	34.9	34.8		34.8	
C-2	32.6[c]	30.0	29.9		30.0	
C-3	198.2	77.7	77.7		77.6	
C-4	124.1	33.0	32.8		32.9	
C-5	170.2	45.1	45.1		45.1	
C-6	35.8[b]	129.4	129.4		129.4	
C-7	32.1[c]	131.1	131.0		131.1	
C-8	35.4	38.2	38.2		38.2	
C-9	56.0	52.7	52.6		52.6	
C-10	38.6	34.5	34.5		34.5	
C-11	21.1	20.9	20.9		20.9	
C-12	38.5	38.4	38.4		38.4	
C-13	44.4	47.1	47.1		47.1	
C-14	53.6	49.1	49.0		49.0	
C-15	24.6	23.6	23.6		23.5	
C-16	23.2	31.9	31.9		31.9	
C-17	58.7	85.3	85.3		85.2	
C-18	13.5	11.4	11.4		11.1	
C-19	17.0	14.5	14.5		14.5	
C-20	210.8	82.7	82.7		83.1	
C-21	70.1	18.0	18.0		18.1	
3-*O*-Sugars		(digit.)[d]	(digit.-1)	(digit.-2)	(digit.)	
C-1		102.7	102.5	106.1	102.7	
C-2		70.9	71.5	71.9	70.8	
C-3		85.0	85.4	84.6	85.0	
C-4		68.7	77.7	68.8	68.6	
C-5		71.1	70.4	71.3	71.0	
C-6		17.4	17.1[e]	17.7[e]	17.4	
–OMe		57.2	59.0	57.3[f]	57.2	
20-*O*-Sugars		(digit.)[d]	(digit.)		(digit.)	(glc.)
C-1		105.7	105.7		105.8	105.3
C-2		71.0	71.3		71.8	75.9
C-3		84.8	84.8		85.4	78.3
C-4		68.5	68.5		76.2	71.9
C-5		71.3	71.1		70.5	78.5
C-6		17.3	17.3[e]		17.6	63.1
–OMe		57.1	57.1[f]		58.8	

a) Signal assignments were done based on the 2-dimensional (2D) NMR (^1H–^{13}C COSY) spectra. *b*—*f*) Signal assignments marked *b*—*f*) may be reversed.

column and a silica gel column.

Compound **2** showed the characteristic signals due to 21-methyl protons (δ 1.63, d), a 20-methine proton (δ 4.17, q) and 6,7-olefinic protons (δ 5.33, d and δ 5.44, d) of teikagenin. Since proton signals assignable to two mol of D-digitalose were observed and the molecular formula was suggested to be $C_{35}H_{58}O_{11}$ by fast atom bombardment (FAB) MS, **2** was considered to be a didigitaloside of teikagenin. In the ^{13}C-NMR spectrum, all carbon signals due to two mol of digitalose were observed without a glycosylation shift, and the C-3 and the C-20 signals of teikagenin were shifted downfield. The structure of **2** was thus determined to be taikagenin 3,20-bis-*O*-β-D-digitaloside, and **2** was named teikaside AL-Ic.

On the basis of the $M^- - 1$ peak at *m/z* 813 in the negative FAB-MS, **3** was suggested to have one additional 6-deoxy-3-*O*-methylhexose as compared with **2**. All proton signals due to the three component sugars were assignable in the ^1H–^1H correlation spectroscopy (COSY) measurement, starting with three anomeric proton signals at δ 4.76, 4.86 and 4.85, and the coupling constants of H-1—H-6 indicated the sugars to be D-digitalose. The H-4 signal and neighboring H-3 and 3-*O*-CH_3 signals were shifted downfield in one of the digitalose residues, suggesting the presence of a 1→4 linked digitalosyl moiety. The chemical shifts in the ^{13}C-NMR spectrum were consistent with the ^1H-NMR assignments, showing the presence of two terminal digitalose residues, and a downfield shift of the C-4 signal in the remaining digitalose.

In order to determine the location of the digitalobiose, the difference nuclear Overhauser effect (NOE) measurement was applied. When the signal of H-3α in the aglycone (δ 3.93) was irradiated, NOE was observed at the anomeric proton signal at δ 4.76. The H-4 signal at δ 4.21 in the same digitalose showed NOE with the second anomeric proton signal at δ 4.86. The structure of **3** was therefore determined to be teikagenin 3-*O*-β-D-digitalosyl-(1→4)-β-D-digitalosyl-20-*O*-β-D-digitaloside and **3** was named teikaside BL-Ic.

The molecular formula of **4** was suggested to be $C_{41}H_{68}O_{16}$ by the $M^- - 1$ peak at *m/z* 815 in the negative FAB-MS. Three anomeric proton signals were observed as doublets (J=8 Hz), and two 6-methyl proton signals and two methoxyl proton signals were observed besides the signals due to teikagenin, indicating **4** to be a teikagenin trioside composed of two 6-deoxy-3-*O*-methylhexoses, possibly digitalose, and one hexose. All proton signals in the component sugars were assignable based on the ^1H–^1H COSY results, and the sugars were determined to be two digitalose and one glucose. Since glycosylation shifts were observed at C-3 and C-20 of teikagenin and at C-4 of one of the digitalose residues, the glucose seemed to be linked to 4-OH of one digitalose. The location of the glucose was finally determined by the difference NOE procedure in the same manner as applied in the case of **3**. While the H-3α signal of the aglycone at δ 3.95 showed NOE with one of the anomeric proton signals at δ 4.82, the H-4 signal of the same digitalose at δ 4.08 showed NOE only with the methyl protons of the neighboring methoxyl group, indicating that one digitalose is linked to the 3-OH of teikagenin. When the anomeric proton of the glucose at δ 5.13 was irradiated, NOE was observed at H-4 (δ 4.32) and the methoxyl proton signals in the remaining digitalose. On irradiation of the H-4 signal of this digitalose at δ 4.32, the anomeric proton signal of the glucose showed NOE. The glucose was therefore assigned to the 4-OH position in the digitalose linked to the C-20 hydroxyl group. Compound **4** was determined to be teikagenin 3-*O*-β-D- digitalosyl-20-*O*-β-D-glucosyl(1→4)-β-D-digitaloside and was named teikaside AL-IId.

Compounds **5**—**9** were identified as teikagenin 3-*O*-β-D-digitaloside, and teikasides A-Ia, A-Ib, A-IIa and A-IIc, respectively in comparison with authentic samples obtained from *T. asiaticum*.[2]

Neither cortexone nor teikagenin 20-*O*-digitaloside has been isolated from *T. asiaticum*. It should be noted that cortexone is a defense substance of water beetles, *Dysticus marginalis*.[7]

Experimental

Specific rotation was recorded with a JASCO DIP 360 polarimeter. ^1H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, br s=broad singlet, br d=broad doublet. The following solvent systems were applied for silica gel column chromatography and

TLC: solvent 1, benzene–acetone; solvent 2, EtOAc–hexane; solvent 3, $CHCl_3$–MeOH–H_2O (bottom layer); solvent 4, EtOAc–MeOH–H_2O (top layer or homogeneous layer); solvent 5, CH_3CN–H_2O. Detection of the spots was carried out by spraying diluted H_2SO_4 onto the TLC plates followed by heating the plates. HPLC was carried out on a Waters ALC 200 equipped with Radial Pack C_{18} eluted with solvent 5.

Extraction Air-dried plants of *Trachelospermum liukiuense* (15 kg) collected at Tanegashima in January, 1987, were powdered and percolated with MeOH. The MeOH percolate (60 l) was concentrated to 5 l *in vacuo*, and partitioned with benzene (extract, 12 g) and $CHCl_3$ (150 g), then the water layer was concentrated *in vacuo*. The concentrated solution was then partitioned with BuOH (588 g).

Cortexone (1) The benzene extract was chromatographed on silica gel columns with solvent 1 (10 : 1), solvent 3 (7 : 1 : 3) and then solvent 2 (3 : 1—2 : 1) to yield a solid (**1**), showing a single spot on TLC (solvent 1). The solid was further purified by a reversed-phase column chromatography on an ODS column with solvent 5 (40%) to give pure **1** (12 mg) as a solid, $[\alpha]_D^{26}$ +151.6° (c=0.095, MeOH). UV λ_{max}^{MeOH} nm (ε): 238 (8600). EI-MS m/z: 330.220 (Calcd for $C_{21}H_{30}O_3$: 330.220). ^{1}H-NMR δ: 0.68, 0.99 (3H each, s, H-18, H-19), 4.45, 4.53 (1H each, d, J=19 Hz, H-21a, b), 5.86 (1H, d, J=2 Hz, H-4). On TLC with solvents 1 and 2, the same Rf values were obtained in a parallel run with authentic cortexone. HPLC t_R 11.6 min (solvent 5, 50%). Upon usual acetylation of **1** with pyridine and Ac_2O at room temperature, **1**-monoacetate was obtained and crystallized from hexane–ether to give prisms, mp 150—154 °C, EI-MS m/z: 372.227 (Calcd for $C_{23}H_{32}O_4$: 372.230). On admixture with authentic cortexone acetate, no melting point depression was observed. In parallel with authentic cortexone acetate, the same Rf values were obtained in TLC with solvents 1 and 2.

Isolation of Teikasides The $CHCl_3$ extract was subjected to MCI-gel chromatography (elution with 0%—80% MeOH), and the eluate with 40—60% MeOH was then fractionated by silica gel column chromatographies with solvent 3 (7 : 1 : 1) and solvent 4 (4 : 1 : 5—4 : 1 : 4) to afford **2**—**9** as solids. Yields: **2** (20 mg), **3** (6 mg), **4** (16 mg), **5** (80 mg), **6** (36 mg), **7** (5 mg), **8** (18 mg), **9** (6 mg). Compounds **5**—**9** were identified as teikagenin 3-*O*-β-D-digitaloside, and teikasides A-Ia, A-Ib, A-IIa and A-IIc, respectively, by comparison with the authentic teikasides on TLC and/or HPLC.

Teikaside AL-Ic (2): A solid, $[\alpha]_D^{28}$ −86.5° (c=1.00, MeOH). FAB-MS m/z: 677.387 (Calcd for $C_{35}H_{58}O_{11}$ + Na: 677.388).

Teikaside BL-Ic (3): A solid, $[\alpha]_D^{28}$ −79.1° (c=0.35, MeOH). Negative FAB-MS m/z: 813 (M^- − 1), 653 (M^- − digit. − 1), 491, 377.

Teikaside AL-IId (4): A solid, $[\alpha]_D^{29}$ −74.1° (c=0.80, MeOH). Negative FAB-MS m/z: 815 (M^- − 1), 653 (M^- − glc.), 491, 371.

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