Tanegosides A, B and C, Lignan Glycosides from *Trachelospermum liukiuense*[1)]

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Three new tetrahydrofuranoid-lignan glycosides, tanegosides A, B and C, were isolated from *Trachelospermum liukiuense* HATSUSIMA, along with 8 lignan glycosides already known in *T. asiaticum*, and the structures were elucidated by spectral and chemical methods.

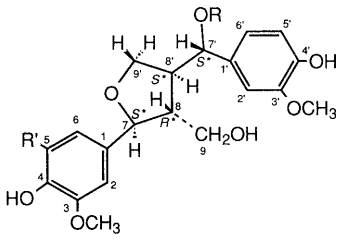
Keywords *Trachelospermum liukiuense*; Apocynaceae; tanegoside; tanegool; lignan; tetrahydrofuranoid-lignan; lignan allopyranoside; lignan glycoside

Two species of *Trachelospermum* (Apocynaceae), *T. asiaticum* NAKAI and *T. liukiuense* HATSUSIMA, are grown in Japan, the former in the main island of Japan and the latter, mostly in the Ryukyu Islands. In the preceding papers of this series, we have described pregnane glycosides,[2)] triterpenoids[3)] and lignans[4)] from *T. asiaticum*, and pregnane glycosides from *T. liukiuense*.[1)] This paper deals with the isolation and structure determination of three new lignan glycosides, tanegosides A, B and C, from *T. liukiuense* along with 8 known lignan glucosides.

When the whole plants of *T. liukiuense* were percolated with MeOH, and the MeOH percolate was partitioned with benzene, $CHCl_3$ and BuOH, the lignan glycosides (**1**—**11**) were obtained from the water layer after partition with BuOH. The isolation of **1**—**11** was carried out by a combination of normal-phase and reversed-phase column chromatographies, and **4**—**11** were identified as known glycosides already obtained from *T. asiaticum*[4,5)]; nortracheloside (**4**), arctigenin gentiobioside (**5**), 4-*O*-glucosyl matairesinoside (**6**), dihydrodehydrodiconiferyl alcohol-4′-*O*-glucoside (**7**), arctiin (**8**), tracheloside (**9**), matairesinoside (**10**) and 8,8′-bisdihydrosyringenin glucoside (**11**).

Tanegoside A (**1**) afforded an $[M+Na]^+$ peak at *m/z* 561.194 in the fast atom bombardment-mass spectrum (FAB-MS), suggesting the molecular formula to be $C_{26}H_{34}O_{12}$. Upon acetylation, **1** afforded a heptaacetate (**1a**), of which two acetyl signals at δ 2.25 and 2.27 were due to phenolic acetyl groups. The sugar moiety of **1** seemed to be glucose, based on the coupling pattern in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum and the signals in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. Upon hydrolysis with β-glucosidase, **1** provided an aglycone (**1b**, solid, $[\alpha]_D$ +64.7°). The degree of unsaturation of **1** was 9, based on the molecular peak observed at *m/z* 376.153 ($C_{20}H_{24}O_7$).

In the ^{1}H–^{1}H chemical shift correlation spectroscopy



1: R=β-D-glucose, R'=H
1a : **1**-heptaacetate
1b : R=R'=H
2 : R=β-D-allose, R'=H
2a : **2**-heptaacetate
3 : R=β-D-glucose, R'=OCH_3

Chart 1

TABLE I. ^{1}H Chemical Shifts of **1**—**3**, **1b** and **1d**, δ (ppm) from Tetramethylsilane (TMS) in Pyridine-d_5 (*J* (Hz) in Parentheses)

H	1	1b	1d	2	3
2	7.39 (d, 2)	7.42 (d, 2)	7.01 (s)	7.40 (d, 2)	7.13 (s)
5	7.19 (d, 8)	7.21 (d, 8)	7.07 (s)	7.19 (d, 8)	
6	7.25 (dd, 8, 2)	7.30 (dd, 8, 2)		7.27 (dd, 8, 2)	7.13 (s)
7	5.19 (d, 7)	5.27 (d, 8)	5.29 (s)	5.20 (d, 8)	5.22 (d, 7)
8	2.62 (m)	2.62 (m)	3.00 (dd, 9, 6)	2.63 (m)	2.68 (m)
9	3.75—3.79	3.84 (dd, 11, 6)	3.70 (dd, 10, 6)	3.75 (dd, 11, 6)	3.71—3.82
	3.82 (dd, 12, 4)	3.90 (dd, 11, 5)	4.00 (dd, 10, 9)	3.83 (dd, 11, 4)	3.87 (dd, 11, 4)
2′	7.53 (d, 2)	7.34 (br s)	7.00 (d, 2)	7.43 (d, 2)	7.53 (d, 2)
5′	7.13 (d, 8)	7.17	7.11 (d, 8)	7.06 (d, 8)	7.14 (d, 8)
6′	7.15 (dd, 8, 2)	7.17	6.74 (dd, 8, 2)	7.14 (dd, 8, 2)	7.18 (dd, 8, 2)
7′	5.49 (d, 8)	5.14 (d, 7)	4.34 (d, 1)	5.47 (d, 8)	5.51 (d, 8)
8′	3.17 (m)	3.15 (m)	2.78 (dd, 6, 1)	3.15 (m)	3.16 (m)
9′α	4.27 (t, 9)	4.41 (dd, 8, 9)	3.90 (br d, 8)	4.29 (t, 9)	4.28 (t, 9)
β	4.81 (dd, 9, 4)	4.89 (dd, 9, 5)	4.24 (dd, 8, 6)	4.84 (dd, 8, 9)	4.83 (dd, 9, 4)
3-OMe	3.71	3.69	3.76	3.72	3.76 (6H, 3,5-OMe)
3′-OMe	3.73	3.68	3.65	3.65	3.73
Sugar moiety					
1″	4.88 (d, 8)			5.33 (d, 8)	4.88 (d, 8)
2″	4.12 (t, 8)			4.09 (dd, 8, 3)	4.12 (t, 8)
3″	4.08 (t, 8)			4.67 (br s)	4.08 (t, 8)
4″	4.18 (t, 8)			4.18 (dd, 9, 2)	4.18 (t, 8)
5″	3.75—3.79			4.26—4.33	3.71—3.82
6″	4.29 (dd, 11, 5)			4.46 (br d, 9)	4.29 (dd, 11, 5)
	4.49 (dd, 11, 2)			4.26—4.33	4.49 (dd, 11, 2)

TABLE II. ^{13}C Chemical Shifts of **1**—**3**, **1b** and **1d**, from TMS in Pyridine-d_5

C	1[a]	1b	1d[a]	2	3
1	134.7	135.2	137.4	134.8	133.8
2	111.2	111.1	111.7	111.3	105.1
3	148.6	148.5	147.3	148.5	149.1
4	147.5	147.3	148.0	147.5	136.9
5	116.2	116.2	119.0	116.2	149.1
6	120.1	119.9	130.1	120.1	105.1
7	84.4	84.4	78.6	84.4	84.5
8	53.1	53.5	45.6	53.0	53.2
9	61.9	62.3	62.2	62.0	62.1
1′	131.6	136.7	133.0	131.9	131.6
2′	112.4	111.5	113.6	112.2	112.4
3′	148.7	148.6	148.5	148.6	148.7
4′	147.8	147.4	146.6	147.7	147.8
5′	116.1	116.1	116.2	116.0	116.1
6′	121.6	120.4	122.2	121.5	121.6
7′	80.8	75.4	53.1	80.8	80.8
8′	49.6	51.2	46.0	49.8	49.7
9′	71.0	71.0	71.2	71.1	71.0
-OMe	55.9	55.8(×2)	55.9(×2)	55.9	56.4(×2)
	55.8			55.7	55.8
Sugar moiety					
1″	100.9			98.7	100.9
2″	75.3			72.4	75.3
3″	78.6			73.0	78.6
4″	72.1			69.6	72.1
5″	78.2			75.6	78.2
6″	63.0			63.3	63.0

a) Signal assignments were done based on the 2D-NMR (^1H–^{13}C and long-range ^1H–^{13}C COSY) spectra.

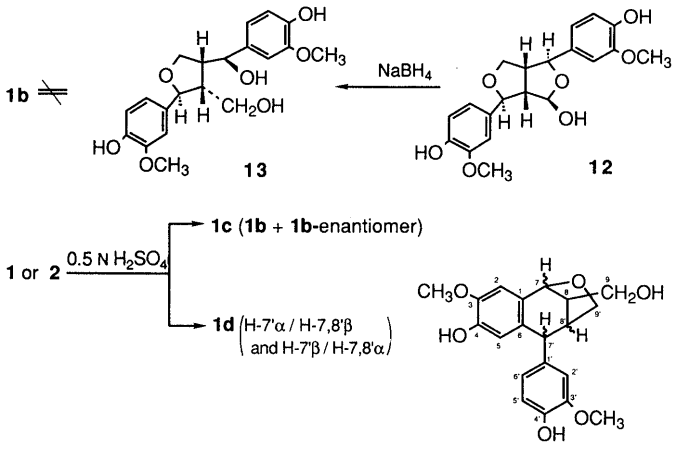


Chart 2

(COSY) spectrum of **1b**, the presence of two 3,4-disubstituted phenyl groups was observed. Since two methoxyl groups showed cross peaks with H-2 and H-2′, respectively, in two dimensional (2D) nuclear Overhauser effect spectroscopy (NOESY), the two phenyl groups were considered to form guaiacyl structures. In the aliphatic region, two three-carbon units, composed of a benzyl carbon bearing oxygen, a methine carbon and a methylene carbon bearing oxygen (C-7, 8, 9; C-7′, 8′, 9′), were connected at the methine carbons (C-8, 8′). The signals in the ^{13}C-NMR spectrum were also consistent with these combinations. Based on the degree of unsaturation, one of the three oxygens in the aliphatic portion of **1b** was considered to form a furan ring. While **1b** seemed to have a similar plane structure to **13**, which was derived from 9-hydroxypinoresinol (**12**),[6] their NMR spectra were not identical with each other. The configurations at C-7, C-8 and C-8′ were assigned as *S*, *R*, *S* or *R*, *S*, *R*, based on the cross peaks between H-7/H-9a, b, 9′α, H-8/H-2, 6, 7′, and H-7′/H-9′β in 2D-NOESY of **1**.

Upon acid hydrolysis, **1** afforded two aglycones, **1c** and **1d**. Compound **1c** showed the same ^1H- and ^{13}C-NMR spectra as those of **1b** but no optical rotation, suggesting **1c** to be a recemate of **1b**. The presence of **1b**-enantiomer in the acid hydrolysate of **1** was well explained by furan ring opening to afford the cation at C-7 and C-7′, and recyclization of the furan ring between the C-7′ cation and C-9-OH. In differential NOE measurements of **1**, H-2′ and H-6′ showed responses as well as H-2,6 and H-7′ on irradiation of H-8, suggesting a closer proximity of the guaiacyl group at C-7′ to C-8 than C-9′, when H-7′ retained β orientation (Chart 1). Therefore, the configuration of C-7′ was determined to be *S* relative to C-7*S*, C-8*R* and C-8′*S*.

Compound **1d** afforded an $[M+Na]^+$ peak at *m/z* 381.131, suggesting the molecular formula $C_{20}H_{22}O_6$, 18 mass units smaller than **1b**, and seemed to be a racemate, based on its low optical rotation value (+1.0°). In the ^1H-NMR spectrum of **1d**, three proton signals due to one of the guaiacyl groups of **1b** were replaced by two *p*-coupled proton signals. The signals of H-7′ and C-7′ showed upfield shifts (−0.80 and −22.3 ppm, respectively) in comparison with those of **1b**. The signal of C-6 was transformed into a singlet from a doublet and shifted downfied (+10.2 ppm). Based on FAB-MS and NMR evidence, the structure of **1d** was assigned as presented in Chart 2. The new C-C linkage seemed to be between C-6 and C-7′ or C-6′ and C-7 in **1b** and **1b**-enantiomer.

The glycosidic linkage of **1** was determined to be at the C-7′ hydroxyl group based on a cross peak between anomeric-H(H-1″)/H-7′ in the 2D-NOESY, and a downfield shift of C-7′ (+5.4 ppm) and upfield shifts of C-1′ (−5.1 ppm) and C-8′ (−1.6 ppm) in comparison with those of **1b**. Compounds **1** and **1b** were named tanegoside A and tanegool, respectively.

Compound **2** has the same molecular formula as **1**. Proton and carbon signals due to the aglycone moiety showed the same chemical shifts and multiplicities as those of **1** and afforded **1c** and **1d** on acid hydrolysis. In the 2D-NOESY of **2**, H-2′ and H-6′ showed cross peaks to H-8, while H-7′ showed cross peaks to H-8, H-9′β and H-1″ as in **1b**. The component sugar was determined to be D-allopyranose ($[\alpha]_D$ +16.0°) based on the ^{13}C-NMR signals of **2** and also direct comparison of the acid hydrolyzate of **2** with authentic D-allose by thin layer chromatography (TLC). Since the β-linkage of the allose moiety was established by the signal of the anomeric proton, the component sugar was assigned as β-D-allopyranose. The structure of the lignan moiety in **2** and the location of the glycosidic linkage were determined to be the same as those of **1**. Compound **2** was named tanegoside B.

The molecular formula of **3** was suggested to be $C_{27}H_{36}O_{13}$ by the molecular peak at *m/z* 591 in the FAB-MS, 30 mass unit (CH_2O) larger than those of **1** and **2**. While the aliphatic region, including the sugar moiety, showed almost the same patterns as in **1** in the NMR spectra, only one guaiacyl group was present in **3**. Instead, two

singlet signals were observed at δ 7.13 (2H) and 3.76 (6H), suggesting that one of the guaiacyl groups was replaced by a syringyl group in **3**. Since a 2H singlet at δ 7.13, assignable to H-2 and H-6, showed a cross peak with H-7 in the 2D-NOESY spectrum, the syringyl group was located at C-7. Based on the glycosylation shift of C-7′ and a cross peak between the anomeric proton and H-7′, the glucosyl linkage was assigned to be at the C-7′ hydroxyl group.

From *T. liukiuense*, arctigenin glucosides such as **5** and **8** were obtained as major lignans, as in *T. asiaticum*. Tanegosides A and B were isolated in the yields of 0.007% and 0.005% from *T. liukiuense* and also below 0.0001% from *T. asiaticum*. Trachelosiaside, a lignan C-glucoside first isolated from *T. asiaticum*,[3] was not observed in *T. liukiuense*.

Experimental

Optical rotations, ^{1}H- and ^{13}C-NMR spectra and MS were obtained as described in the preceding paper.[1] Column chromatography and TLC were carried out with the following solvent systems: solvent 1, EtOAc–MeOH–H_2O (top layer or homogeneous layer); solvent 2, $CHCl_3$–MeOH–H_2O (bottom layer); solvent 3, CH_3CN–H_2O.

Isolation of Tanegosides Extraction of *Trachelospermum liukiuense* HATSUSIMA (air-dried, 15 kg) and fractionation with benzene, $CHCl_3$ and BuOH were described in the preceding paper.[1] After partition with BuOH, the H_2O layer was concentrated *in vacuo* and then passed through an MCI-gel (Mitsubishi Chem. Co., CHP-20P) column. The column was eluted with H_2O, and then H_2O containing MeOH. The eluate with 25% MeOH was chromatographed on a silica gel column with solvents 1 (4:1:3—3:1:3) and 2 (7:3:2—7:3:1.2) and an RQ-1 column (Fuji gel) with solvent 3 (15%) to afford **1** (1.072 g), **2** (814 mg), **3** (62 mg), **4**, (18 mg), **5** (28 mg), **6** (138 mg) and **7** (13 mg). The eluate with 50% MeOH was chromatographed on silica gel columns with solvents 1 (4:1:4—4:1:3) and 2 (7:2:1—7:3:1) to afford **5** (1.12 g), **8** (*ca.* 4.0 g), **9** (280 mg), **10** (940 mg) and **11** (87 mg). Compounds **4**—**11** were identified by direct comparisons with the authentic samples from *T. asiaticum*.[4]

Air-dried plants of *T. asiaticum* (2.4 kg) collected in the Fukuoka district in November, 1988, were treated according to the same procedure as described above. From the water layer **1** (8 mg) and **2** (9 mg) were isolated.

Tanegoside A (1) A solid, $[\alpha]_D^{31}$ −20.0° (c=2.53, MeOH). FAB-MS m/z 561.194 (Calcd for $C_{26}H_{34}O_{12}$+Na: 561.195). 2D-NOESY cross peaks: H-2/3-OMe, H-7, 8; H-6/H-7, 8; H-7/H-9a, b, 9′α; H-8/H-7′, 2′; H-2′/3′-OMe, H-7′, 8′, 1″; H-6′/H-7′, 8′; H-7′/H-9′β, 1″; H-1″/H-3″, 5″. Upon usual acetylation of **1** with Ac_2O and pyridine at room temperature, **1**-heptaacetate (**1a**) was obtained, FAB-MS m/z: 855 ($C_{40}H_{48}O_{19}$+Na), ^{1}H-NMR δ: 1.90, 1.98, 2.01, 2.02, 2.06, 2.25, 2.27 (3H each, s, OAc), 2.46, 2.76 (1H each, m, H-8, 8′), 3.77, 3.82 (3H each, s, OMe), 4.78 (1H, d, J=8 Hz, H-1″), 4.95 (1H, d, J=8 Hz, H-7), 5.13 (1H, d, J=8 Hz, H-7′), 7.08, 7.19 (1H each, dd, J=8, 1 Hz, H-6, 6′), 7.23, 7.33 (1H each, d, J=1 Hz, H-2, 2′), 7.24, 7.29 (1H each, d, J=8 Hz, H-5, 5′).

Compound **1** (45 mg) was dissolved in 20% EtOH (4 ml) and the solution was shaken with cellulase (Sigma Chem. Co., Ltd.) (30 mg) at 38 °C for 15 h. The mixture was extracted with BuOH and the BuOH extract was purified on a silica gel column with solvent 2 (7:2:1) to give **1b** (tanegool) (11 mg) as a solid, $[\alpha]_D^{29}$ +64.7° (c=0.45, MeOH), EI-MS m/z: 376.153 (Calcd for $C_{20}H_{24}O_7$: 376.152). 2D-NOESY cross peaks: H-2/3-OMe, H-7; H-6/H-7; H-7/H-9′α, 9a, b; H-2′/3′-OMe, H-7′, 8′; H-6′/H-7′, 8′; H-7′/H-8, 9′β.

Compound **1** (210 mg) was refluxed with 0.5 N H_2SO_4 in 50% dioxane (4 ml) for 30 min. The mixture was diluted with MeOH and deacidified with IRA-410. The methanolic solution was then concentrated *in vacuo*, diluted with H_2O and extracted with BuOH. The BuOH extract was chromatographed on a silica gel column with solvent 2 (7:1:2) to give **1c** (11 mg) and **1d** (45 mg), each as a solid. **1c**: $[\alpha]_D^{29}$ +0.4° (c=0.9, MeOH). **1c** showed the same *Rf* value and NMR spectra as those of **1b**. **1d**: $[\alpha]_D^{28}$ +1.0° (c=1.5, MeOH), FAB-MS m/z: 381.131 (Calcd for $C_{20}H_{22}O_6$+Na: 381.131). 2D-NOESY cross peaks: H-2/H-7; H-8/H-2′, 6′; H-9a/H-9′β; H-7′/H-5, 9′α. D-Glucose was detected in the H_2O layer.

Tanegoside B (2) A solid, $[\alpha]_D^{30}$ −24.7° (c=1.94, MeOH), FAB-MS m/z: 561.195 (Calcd for $C_{26}H_{34}O_{12}$+Na: 561.195). 2D-NOESY cross peaks: H-2/3-OMe, H-7, 8; H-6/H-7, 8; H-7/H-9a, b, 9′α; H-8/H-7′, 2′, 6′; H-2′/3′-OMe, H-7′, 8′, 1″; H-6′/H-7′, 8′, 1″; H-7′/H-9′β, 1″; H-1″/H-5″. A heptaacetate of **2** (**2b**) was obtained by acetylation with Ac_2O and pyridine as a solid, FAB-MS m/z: 855 ($C_{40}H_{48}O_{19}$+Na). ^{1}H-NMR δ: 1.92, 1.94, 1.98, 2.02, 2.05 (3H each, s, OAc), 2.25 (6H, s, 2×OAc), 2.57 (1H, m, H-8), 2.79 (1H, m, H-8′), 3.77, 3.82 (3H each, s, OMe), 4.06 (1H, dd, J=9, 7 Hz, H-9′α), 4.11 (1H, dd, J=12, 5 Hz, H-9a), 4.17 (1H, dd, J=12, 6 Hz, H-9b), 4.34 (1H, dt, J=10, 3 Hz, H-5″), 4.50 (2H, br s, H-6″), 4.68 (1H, dd, J=9, 5 Hz, H-9′β), 4.81 (1H, d, J=8 Hz, H-7), 5.240 (1H, d, J=6 Hz, H-7′), 5.243 (1H, d, J=8 Hz, H-1″), 5.41 (1H, dd, J=10, 3 Hz, H-4″), 5.44 (1H, dd, J=10, 3 Hz, H-2″), 6.17 (1H, t, J=3 Hz, H-3″), 7.12, 7.23 (1H each, dd, J=8, 1 Hz, H-6, 6′), 7.25 (2H, d, J=8 Hz, H-5, 5′), 7.28, 7.35 (1H each, br s, H-2, 2′).

Compound **2** (80 mg) was treated with 0.5 N H_2SO_4 in 50% dioxane in the same manner as described above. From the BuOH extract, **1c** (4 mg) and **1d** (13 mg) were obtained. The H_2O layer was concentrated *in vacuo* and the residue was passed through an RQ-1 column. The eluate with H_2O gave D-allose, $[\alpha]_D^{24}$ +16.0 (c=1.2, H_2O).

Tanegoside C (3) A solid, $[\alpha]_D^{29}$ −32.6° (c=1.5, MeOH), FAB-MS m/z: 591 ($C_{27}H_{36}O_{13}$+Na). 2D-NOESY cross peaks: H-2, 6/3, 5-OMe, H-7, 8; H-7/H-9a, b, 9′α; H-8/H-7′; H-2′/3′-OMe, H-7′, 8′, 1″; H-6′/H-7′; H-7′/H-9′β, 1″; H-1″/H-3″, 5″.

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