New C_{28} Steroidal Glycosides from *Tubocapsicum anomalum*

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Two new C_{28} steroidal glycosides, tuboanosides A (1) and B (2), were isolated from the fruit of *Tubocapsicum anomalum* MAKINO. Their chemical structures were elucidated on the basis of spectroscopic and X-ray diffraction analysis of the *p*-bromobenzoyl derivative (4) of tuboanosigenin (3), the sapogenol derivative of these two glycosides. Tuboanosides have the structural peculiarity of an unusual side chain carrying an unusual linkage with a C-21 bound to C-25 on the lactone ring.

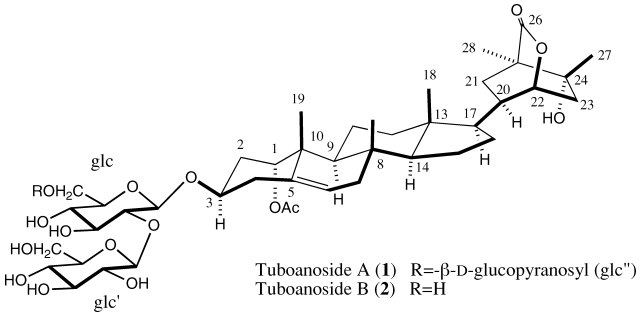
Key words *Tubocapsicum anomalum*; C_{28} steroidal glycoside; X-ray analysis

Tubocapsicum anomalum is a plant widely distributed in China, India, the Philippines, and Japan. It has antigonorrhel, antifuruncular, antiinflammatory, and antiintumescent effects.[1)] Previously, as a part of studies on the steroidal glycosides in solanaceous plants, two novel C_{28} steroidal lactone glycosides, tubocaposides A and B, were isolated from the fresh fruit of *T. anomalum* and their structures determined using X-ray diffraction analysis.[2)] Following that study, we also reported isotubocaposides A, B, and C from the fresh fruit of this plant.[3)]

Continuing research on this plant led to the isolation of two new steroidal glycosides, tuboanosides A (**1**) and B (**2**). They were isolated from the MeOH extract of the aerial parts of *T. anomalum* by a combination of high porous resin (Diaion HP-20), and normal- and reversephase silica gel column chromatography.

The sapogenol moiety of these two glycosides was also regarded as an ergostane derivative by comparison with the NMR spectrum of tuboanosigenin (**3**) obtained by enzymatic hydrolysis of tuboanoside A (**1**). The sapogenol in turn was derivatized into a *p*-bromobenzoyl derivative (**4**) to afford colorless prisms. The derivative was subjected to X-ray analysis to establish its structure. This report describes the structural characterization of tuboanosides A (**1**) and B (**2**).

Tuboanoside A (**1**), obtained as an amorphous powder, showed $[\alpha]_D$ −32.5° (MeOH), and the molecular formula $C_{48}H_{74}O_{21}$ with positive-ion FAB-MS. The ^{1}H-NMR spectrum of **1** displayed four *tert*-methyls at δ 0.56, 1.08, 1.47, and 1.53, an acetyl group at δ 2.06, an acetoxy-bearing methine proton at δ 5.32 (1H, d, J=7.9 Hz), an olefinic proton at δ 5.55 (1H, br s), and three anomeric protons at δ 5.01 (1H, d, J=7.9 Hz), 5.05 (1H, d, J=7.9 Hz), and 5.22 (1H, d, J=7.9 Hz). On the other hand, the ^{13}C-NMR signals (Table 1) displayed two terminal glucopyranosyl moieties[4)] (glc′ and glc″) at δ 105.4, 106.7 (2×C-1), 75.2, 76.8 (2×C-2), 78.7, 78.1 (2×C-3), 71.8, 71.7 (2×C-4), 78.4, 78.3 (2×C-5), 62.8, and 63.0 (2×C-6) and a 2,6-di-*O*-sugar-substitued glucopyranosyl moiety[4)] (glc) at δ 101.0 (C-1), 84.6 (C-2), 77.6 (C-3), 71.3 (C-4), 76.9 (C-5), and 69.9 (C-6). After deducting these signals originating from sugar, the remainder totals 30 carbon signals including one acetyl group. Therefore the sapogenol was regarded as an ergostane derivative, which comprised four oxygen-bearing carbons at δ 71.0, 74.3, 75.3, and 77.8, one lactone carbonyl group at δ 177.8, and one acetyl group at δ 21.1 and 170.5. The structure of this new sapogenol was deduced to be similar to that of tubocaposide,[2)] although there were some differences in the substitution moiety at C-17 in the ^{1}H- and ^{13}C-NMR signals.



Tuboanoside A (**1**) R=-β-D-glucopyranosyl (glc'')
Tuboanoside B (**2**) R=H

Enzymatic hydrolysis[5)] of tuboanoside A (**1**) by glycosidases from *Turbo cornutus* provided tuboanosigenin (**3**), obtained as an amorphous powder, showing $[\alpha]_D$ −26.9° (MeOH). HR-FAB-MS showed the molecular formula $C_{30}H_{44}O_6$. The ^{1}H-NMR spectrum of **3** showed four *tert*-methyls at δ 0.67, 1.08, 1.12, and 1.27, an acetyl at δ 2.04, three oxygen-bearing methine protons at δ 3.87 (1H, m), 4.39 (1H, br s), and 5.06 (1H, br s), and an olefinic proton at 5.51 (1H, br s). The ^{13}C-NMR spectrum exhibited 30 carbons, which were comprised of three oxygen-bearing methane carbons at δ 71.9, 75.1, and 77.4, a trisubstituted double bond at δ 124.1 and 137.3, a lactone carbonyl group at δ 170.2, and an acetyl group at δ 21.1 and 170.2. The HMBC (Fig. 1) of **3** enabled the assignment of ^{1}H- and ^{13}C-NMR signals to provide a planar structure for **3**.

Next, the sapogenol (**3**) was derivatized into a *p*-bromobenzoate (**4**) by reaction with *p*-bromobenzoyl chloride and pyridine at room temperature.[6)] The benzoate obtained as colorless prisms, mp 162—163 °C, $[\alpha]_D$ −31.9° ($CHCl_3$), had the molecular formula $C_{37}H_{47}BrO_7$ based on positive-ion FAB-MS. The ^{1}H-NMR spectrum of **4** showed a multiplet signal at δ 5.45 down-shifted by 1.58 ppm from that of **3** and A_2B_2 aromatic proton signals at δ 7.71, 8.06 (each 2H, d, J= 8.6 Hz). Therefore **4** was characterized as a 3-*O*-*p*-bromobenzoate of tuboanosigenin. The X-ray analysis of **4** is shown in Fig. 2.

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Table 1. ^{13}C-NMR Data for Tuboanosides A (**1**), B (**2**), Tuboanosigenin (**3**), and *p*-Bromobenzoate of **3** (**4**) (C_5D_5N, 500 MHz)

C	**1**	**2**	**3**	**4**
1	75.3	75.3	75.1	74.8
2	34.4	34.5	35.7	32.2
3	74.3	74.0	66.7	70.9
4	38.3	38.4	41.5	37.7
5	137.8	137.8	137.3	136.6
6	124.2	124.2	124.1	125.5
7	32.0	32.0	31.6	31.9
8	32.0	32.0	31.9	32.0
9	42.4	42.4	42.3	42.3
10	41.0	41.0	40.4	40.9
11	20.6	20.6	20.4	20.5
12	39.5	39.5	39.4	39.4
13	43.0	42.9	42.9	43.0
14	55.5	55.5	55.9	55.4
15	24.6	24.6	24.4	24.6
16	26.8	26.8	26.9	26.8
17	52.7	52.8	52.7	52.7
18	12.9	12.9	13.0	12.9
19	19.6	19.6	19.5	19.4
20	39.8	39.8	39.0	39.8
21	31.7	31.7	31.1	31.7
22	77.8	77.8	77.4	77.8
23	39.4	39.4	38.0	39.4
24	71.0	71.0	71.9	71.0
25	48.2	48.2	47.3	48.2
26	177.8	177.8	177.2	177.8
27	15.2	15.2	14.2	15.2
28	29.0	20.0	29.1	20.0
–OAc	170.5	170.1	170.2	170.2
	21.1	20.9	21.1	20.9
glc-1	101.0	101.1		
-2	84.6	84.9		
-3	77.6	77.8		
-4	71.3	71.5		
-5	76.9	77.1		
-6	69.9	62.5		
glc′-1	106.7	106.8		
-2	76.8	75.3		
-3	78.1	78.8		
-4	71.7	71.8		
-5	78.3	78.2		
-6	63.0	63.0		
glc″-1	105.4			
-2	75.2			
-3	78.7			
-4	71.8			
-5	78.4			
-6	62.8			
2′, 6′				132.2
3′, 5′				131.6

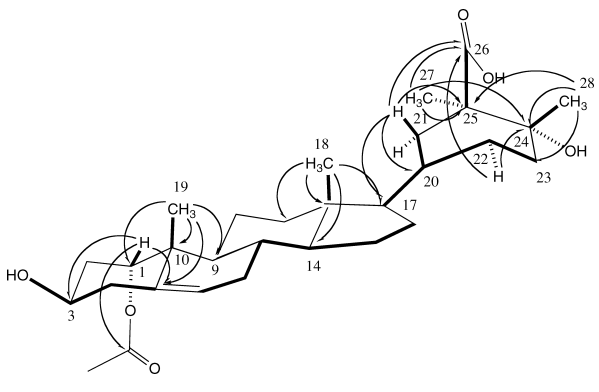

Fig. 1. Key HMBC Correlation of Tuboanosigenin (**3**)

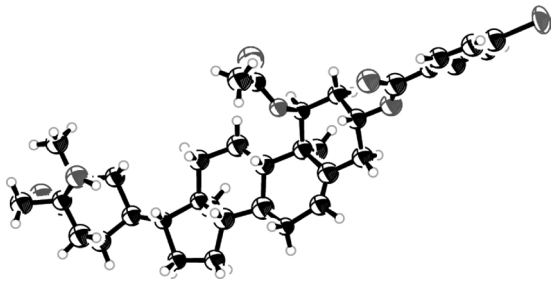

Fig. 2. ORTEP Drawing of Tuboanosigenin *p*-Bromobenzoate (**4**)

As mentioned above, although the sugar linkage was deduced based on the ^{13}C-NMR data, it was additionally verified based on the HMBC spectrum. That is, correlations were observed from each terminal glucopyranosyl anomeric proton at δ 5.01 and 5.22 to C-2 at δ 84.6 and C-6 at δ 69.9 of the inner glucopyranosyl moiety, as well as from the inner glucopyranosyl anomeric proton to the C-3 at δ 74.3 of the sapogenol. Therefore the structure of tuboanoside A (**1**) could be expressed as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl tuboanosigenin.

Tuboanoside B (**2**), obtained as an amorphous powder, exhibited $[\alpha]_D$ −26.5° (MeOH) and the molecular formula $C_{42}H_{64}O_{16}$ in positive-ion FAB-MS. The ^{1}H-NMR spectrum of **2** showed signals due to four *tert*-methyls at δ 0.56, 1.10, 1.47, and 1.53, an acetyl at δ 1.90, two anomeric protons at δ 5.12 (1H, d, J=7.9 Hz) and 5.28 (1H, d, J=7.9 Hz), an acetoxy-bearing methine proton at δ 5.25 (1H, br s), and an olefinic proton at δ 5.50 (1H, br s). The ^{13}C-NMR spectrum displayed a total of 42 carbon signals as listed in Table 1. They were comprised of three oxygen-bearing methine carbons at δ 74.0, 75.3, and 77.8, an oxygen-bearing quaternary carbon at δ 71.0, a trisubstituted double bond at δ 137.8 and 124.2, a lactone carbonyl at δ 177.8, an acetyl group at δ 20.9 and 170.1, and two anomeric carbon signals at δ 101.1 and 106.8. The ^{13}C-NMR spectrum of **2** indicated the presence of a terminal β-D-glucopyranosyl moiety and a 6-*O*-glycosylated β-D-glucopyranosyl moiety.[4] Hence the structure of tuboanoside B (**2**) was determined to be 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl tuboanosigenin.

Tuboanosides have the structural peculiarity of an unusual side chain carrying a C-21 bound to the C-25 on the lactone ring.

Experimental

General Experimental Procedures Column chromatography was carried out with Diaion HP-20 and MCI gel CHP20P (Mitsubishi Kagaku, Tokyo, Japan), silica gel 60 and 60N (Kanto Chemical Co., Inc., Tokyo, Japan), and Chromatorex ODS (Fuji Silysia Chemical Co., Ltd.). HPLC was performed on ODS (Cosmosil 5C_{18}-MS-II; Nacalai Tesque Co., Ltd., Kyoto, Japan; Φ=20 mm, L=250 mm), and the eluate was monitored with an RI detector. TLC was performed on precoated silica gel 60 F_{254} (Merck) and RP-18 F_{254}S (Merck) plates. Melting points were measured with a Yanagimoto micromelting apparatus.

Optical rotations were measured using a Jasco DIP-1000KUY polarimeter (l=0.5). NMR spectra were measured in C_5D_5N with a Jeol α-500 spectrometer, and chemical shifts were referenced to TMS. Positive-ion HR-FAB-MS spectra were recorded on a JEOL JMS-DX303HF spectrometer.

Plant Material Fruit (650 g) of *T. anomalum* were collected in Kabutoiwa, Uto, Kumamoto, Japan, in November 2003, and voucher specimens are deposited in the Laboratory of Natural Medicines, Kumamoto University.

Extraction and Isolation Air-dried fruits (650 g) of *T. anomalum* (650 g) were extracted twice with MeOH (2.1 l) to yield the MeOH-soluble

fraction (56.1 g). The MeOH-soluble fraction was applied to highly porous resin (Diaion HP-20) with H_2O, MeOH, and acetone, and their respective fractions were collected. The residue (9.76 g) of the MeOH eluate was applied to silica gel (350 g) with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5) and fractionated into fractions (fr.) 1—7. The residue (2.26 g) of fr. 4 was applied to Chromatorex ODS (200 g) with 60% MeOH to give 250 mg of tuboanoside A (**1**). Fr. 2 (1.00 g) was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (20 : 1)—$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1—8 : 2 : 0.2) and on ODS with 60% MeOH to provide tuboanoside B (**2**, 6.6 mg).

Tuboanoside A (**1**): An amorphous powder, $[\alpha]_D^{25}$ −32.5° (c=0.1, MeOH). Positive FAB-MS (m/z): 1009 $[M+Na]^+$, HR-FAB-MS (m/z): 1009.4632 (Calcd for $C_{48}H_{74}O_{21}$ 1009.4620). ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.56 (3H, s, H_3-18), 1.08 (3H, s, H_3-19), 1.47 (3H, s, H_3-28), 1.53 (3H, s, H_3-27), 2.06 (3H, s, Ac), 4.74 (1H, d, J=11.0 Hz, glc H-6), 5.01 (1H, d, J=7.9 Hz, glc′ H-1), 5.05 (1H, d, J=7.9 Hz, glc H-1), 5.22 (1H, d, J=7.9 Hz, glc″ H-1), 5.55 (1H, br s, H-6). ^{13}C-NMR (C_5D_5N, 500 MHz) δ: see Table 1.

Tuboanoside B (**2**): An amorphous powder, $[\alpha]_D^{25}$ −26.5° (c=0.1, MeOH). Positive FAB-MS (m/z) 847 $[M+Na]^+$, HR-FAB-MS (m/z) 847.4077 (Calcd for $C_{42}H_{64}O_{16}$ 847.4092). ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.56 (3H, s, H_3-18), 1.10 (3H, s, H_3-19), 1.47 (3H, s, H_3-28), 1.53 (3H, s, H_3-27), 1.90 (3H, s, Ac), 5.12 (1H, d, J=7.9 Hz, glc H-1), 5.25 (1H, br s, H-1), 5.12 (1H, d, J=7.9 Hz, glc H-1), 5.25 (1H, br s, H-1), 5.28 (1H, d, J=7.9 Hz, glc′ H-1), 5.50 (1H, br s, H-6). ^{13}C-NMR (C_5D_5N, 500 MHz) δ (Table 1).

Enzymatic Hydrolysis of Tuboanoside A (1) Tuboanoside A (**1**) (140 mg) in 3 ml of DMSO was enzymatically hydrolyzed with mixed glycosidases from *Turbo cornutus* at 37 °C for 48 h. The reaction mixture was extracted with MeOH. The residue of the MeOH extract was applied to highly porous synthetic resin (Diaion HP-20) with H_2O and MeOH, and their respective fractions were collected. The residue (213 mg) of the MeOH eluate was applied to silica gel (50 g) with n-hexane–acetone (4 : 1) to give 32.6 mg of tuboanosigenin (**3**).

Tuboanosigenin (**3**): An amorphous powder, $[\alpha]_D^{25}$ −26.9° (c=0.1, $CHCl_3$). Positive FAB-MS (m/z): 523.3076 $[M+Na]^+$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.67 (3H, s, H_3-18), 1.08 (3H, s, H_3-19), 1.12 (3H, s, H_3-27), 1.27 (3H, s, H_3-28), 2.04 (3H, s, Ac), 3.87 (1H, m, H-3), 4.39 (1H, br s, H-22), 5.06 (1H, br s, H-1), 5.51 (1H, br s, H-6). ^{13}C-NMR (C_5D_5N, 500 MHz) δ see Table 1.

Preparation of Tuboanosigenin *p*-Bromobenzoate (4) A solution of tuboanosigenin (**4**) (20.3 mg) in pyridine (500 μl) was treated with p-bromobenzoyl chloride (150 mg) in CH_2Cl_2 (4 ml) at room temperature for 24 h. The residue of the reaction mixture was applied to silica gel (20 g) with n-hexane–acetone (10 : 1→9 : 1→8 : 1) to give 12.5 mg of tuboanosigenin p-bromobenzoate (**4**).

Tuboanosigenin p-Bromobenzoate (**4**): Colorless prism, mp 162—163 °C, $[\alpha]_D^{25}$ −31.9° (c=0.1, $CHCl_3$). Positive FAB-MS (m/z): 705 $[M+Na]^+$. ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.60 (3H, s, H_3-18), 1.10 (3H, s, H_3-19), 1.48 (3H, s, H_3-28), 1.53 (3H, s, H_3-27), 2.09 (3H, s, Ac), 4.53 (1H, br s, H-22), 5.30 (1H, br s, H-1), 5.45 (1H, m, H-3), 5.57 (1H, br s, H-6), 7.71 (2H, d, J=8.5 Hz), 8.06 (2H, d, J=8.6 Hz).

X-Ray Analysis of 4 Reflection data were obtained using a Rigaku RAXIS-RAPID diffractometer with molybdenum radiation Mo$K\alpha$ (λ=0.71075 Å). All diagrams and calculations were performed using Crystal Structure[7]: Crystal dimension=0.3×0.2×0.5 mm, triclinic space group P1, a=11.639(2) Å, b=15.415(2) Å, c=16.296(3) Å, V=1693.2(5) Å^3, Z=1, D_{calc}=1.341 g/cm^3, 5113 observed reflections, final residuals R with 0.139 (this value was not highly reliable probably owing to tiny crystal size), CCDC reference number 680092.

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