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

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Received June 28, 2006; accepted September 15, 2006

Three new C_{28} steroidal glycosides, isotubocaposides A (1), B (2), and C (3), were isolated from the fruits of *Tubocapsicum anomalum* MAKINO. Their chemical structures were elucidated on the basis of spectroscopic and X-ray diffraction analysis of *p*-bromobenzoyl derivative (5) of isotubocaposigenin (4), the sapogenol derivative of these three glycosides. Isotubocaposides have the structural peculiarity of an unusual side chain carrying a C-21 bound to C-24 on the lactone ring.

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

Tubocapsicum anomalum is a plant widely distributed in China, India, the Philippines, and Japan. It possesses anti-gonorrhea, anti-furuncle, anti-inflammation, and anti-intumescent effects. Previously, as a part of studies on the steroidal glycosides in solanaceous plants, two novel C_{28} steroidal lactone glycosides named tubocaposides A and B were isolated from the fresh fruits of *T. anomalum* and their structures determined by X-ray diffraction analysis.[1)]

Continuing research on this plant has led to the isolation of three new steroidal glycosides, named isotubocaposides A (**1**), B (**2**), and C (**3**). They were isolated from the MeOH extract of the fruits of *T. anomalum* by combination of high porous resin (Diaion HP-20), and normal- and reversed-phase silica gel column chromatographies.

The sapogenol, isotubocaposigenin (**4**), of these three glycosides was regarded as identical by comparing their NMR spectra. Isotubocaposigenin (**4**) was obtained by enzymatic hydrolysis of isotubocaposide A (**1**). The sapogenol in turn was derivatized into a *p*-bromobenzoyl derivative (**5**) to afford colorless plates. The derivative was subjected to X-ray analysis to establish the structure. This report describes the structural characterization of isotubocaposides A (**1**), B (**2**), and C (**3**).

Isotubocaposide A (**1**), obtained as an amorphous powder, $[\alpha]_D$ −26.5° (MeOH), showed a molecular formula $C_{48}H_{74}O_{21}$ by high resolution (HR)-FAB-MS. The ^{1}H-NMR spectrum of **1** displayed four *tert*-methyls at δ 0.53, 1.09, 1.44, 1.77, an acetyl at δ 2.10, three anomeric protons at δ 5.02 (1H, d, J=7.9 Hz), 5.06 (1H, d, J=7.3 Hz), 5.22 (1H, d, J=7.3 Hz), an acetoxy-bearing methine proton at δ 5.32 (1H, br s), and an olefinic proton at δ 5.57 (1H, br s). On the other hand, the ^{13}C-NMR signals (Table 1) displayed three oxygen-bearing methine carbons at δ 75.3, 74.3, 85.1, an oxygen-bearing quaternary carbon at δ 77.0, a tri-substituted double bond at δ 124.2, 137.8, a lactone carbonyl at δ 175.2, an acetyl group at δ 21.1, 170.5, and three anomeric carbons at δ 101.0, 105.3, 106.7.

Enzymatic hydrolysis of isotubocaposide A (**1**) by glycosidases from *Turbo cornutus* provided isotubocaposigenin (**4**), obtained as an amorphous powder, $[\alpha]_D$ −42.2° (MeOH). The HR-FAB-MS afforded a molecular formula $C_{30}H_{44}O_6$. The ^{1}H-NMR spectrum of **4** showed four *tert*-methyls at δ 0.57, 1.10, 1.44, 1.77, an acetyl at δ 2.09, three oxygen-bearing methine protons at δ 4.34 (1H, m), 4.59 (1H, br s), 5.34 (1H, br s), and an olefinic proton at 5.59 (1H, br s). The ^{13}C-NMR exhibited 30 carbons, which were constituted with an oxygen-bearing quaternary carbon at δ 77.0, a tri-substituted double bond at δ 123.7, 138.7, a lactone carbonyl group at δ 175.2, an acetyl group at δ 21.0, 170.2. The HMBC (Fig. 1) of **4** enabled the assignment of ^{1}H- and ^{13}C-NMR signals to provide a plane structure for **4**, which was identical with that of tubocaposigenin.[1)]

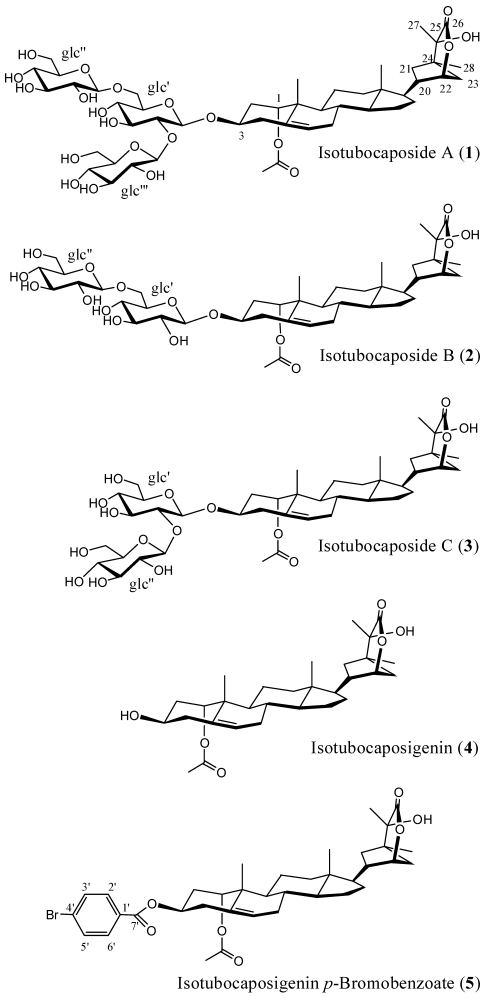

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Table 1. ^{13}C-NMR Data for Isotubocaposides A (**1**), B (**2**), and C (**3**), Isotubocaposigenin (**4**), and Isotubocaposigenin *p*-Bromobenzoate (**5**) (C_5D_5N, 500 MHz)

C	1	2	3	4	5
1	75.3	75.2	75.3	75.7	74.8
2	34.4	34.3	34.5	36.5	32.3
3	74.3	73.2	74.1	65.9	71.0
4	38.3	38.5	38.4	42.7	37.8
5	137.8	137.6	137.8	138.7	136.6
6	124.2	124.5	124.2	123.7	125.5
7	31.9	31.9	32.0	32.0	31.9
8	32.0	32.0	32.0	32.0	31.9
9	42.6	42.6	42.6	42.6	42.5
10	41.0	41.0	41.0	40.8	40.9
11	20.7	20.7	20.7	20.7	20.7
12	39.6	39.6	39.6	39.6	39.6
13	42.7	42.7	42.7	42.6	42.7
14	56.1	56.1	56.1	56.2	56.0
15	24.6	24.6	24.6	24.6	24.6
16	28.0	28.0	28.0	28.0	28.0
17	54.8	54.8	54.8	54.8	54.8
18	12.9	12.9	12.9	12.9	12.9
19	19.6	19.5	19.6	19.6	19.5
20	49.4	49.4	49.4	49.4	49.4
21	37.8	37.7	37.8	37.7	37.7
22	85.1	85.2	85.1	85.1	85.0
23	39.5	39.5	39.5	39.5	39.6
24	47.6	47.6	47.6	47.6	47.7
25	77.0	77.0	77.0	77.0	77.0
26	175.2	175.3	175.2	175.2	175.1
27	20.1	20.1	20.1	20.1	20.1
28	20.6	20.6	20.6	20.6	20.6
–OAc	170.5	170.6	170.1	170.2	170.2
	21.1	21.2	20.9	21.0	20.9
glc′ -1	101.0	102.3	101.1		
-2	84.6	75.2	84.9		
-3	77.6	78.4	77.9		
-4	71.2	71.6	71.4		
-5	76.9	77.1	77.0		
-6	69.9	70.1	62.5		
glc″ -1	105.3	105.3	106.7		
-2	75.2	75.1	75.3		
-3	78.6	78.4	78.7		
-4	71.7	71.5	71.7		
-5	78.4	78.3	78.2		
-6	62.9	62.7	63.0		
glc‴ -1	106.7				
-2	76.7				
-3	78.0				
-4	71.7				
-5	78.3				
-6	62.8				
2′, 6′					132.2
3′, 5′					131.6

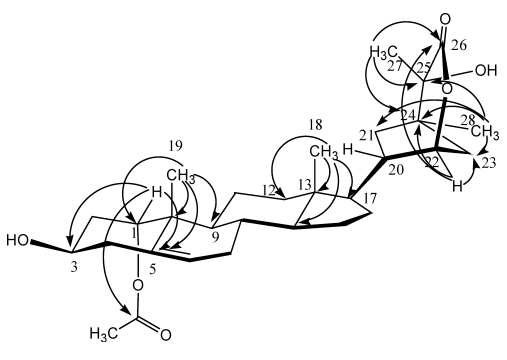

Fig. 1. Key HMBC Correlation of Isotubocaposigenin (**4**)

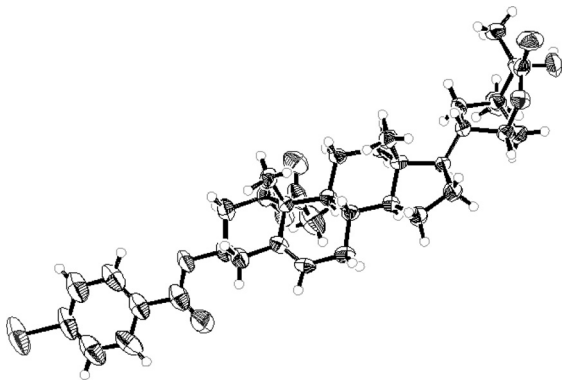

Fig. 2. X-Ray Analysis of Isotubocaposigenin *p*-Bromobenzoate (**5**)

The sapogenol (**4**) was subsequently derivatized into a *p*-bromobenzoate (**5**) by the reaction with *p*-bromobenzoyl chloride and pyridine at room temperature.[2] The benzoate obtained as colorless plates, mp 152—154 °C, $[\alpha]_D$ −31.9° ($CHCl_3$), gave a molecular formula $C_{37}H_{47}BrO_7$ by HR-FAB-MS. The ^{1}H-NMR spectrum of **5** showed a multiplet signal at δ 5.46 being lower-shifted by 1.12 ppm from that of **4** and A_2B_2 aromatic proton signals at δ 7.71, 8.06 (each 2H, d, J=8.5 Hz). Therefore **5** was characterized as 3-*O*-*p*-bromobenzoate of isotubocaposigenin. Reflection data were obtained by Rigaku RAXIS-RAPID diffractometer using molybdenum radiation Mo$K\alpha$ (λ=0.71075 Å). All diagrams and calculations were performed using Crystal Structure[3]: Crystal dimension=0.6×0.6×0.1 mm, orthorhombic space group $P2_12_12_1$, a=7.192 (2) Å, b=11.497 (3) Å, c=40.752 (8) Å, v=3369 (1) Å^3, Z=4, D_{calc}=1.348 g/cm^3, $D_{observe}$=1.327 g/cm^3, 7718 observed reflections, final residuals R and R_1 with 0.141 and 0.094, respectively. Consequently, the structure of **5** was determined as shown in Fig. 2. Hence isotubocaposigenin (**4**) is a stereo-isomer at C-25 of tubocaposigenin.

As regards the sugar linkage, the ^{13}C-NMR spectrum of **1** disclosed the occurrence of two terminal β-D-glucopyranosyl moieties and a 2,6-di-*O*-glycosylated β-D-glucopyranosyl moiety. Therefore the structure of isotubocaposide A was expressed as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl isotubocaposigenin.

Isotubocaposide B (**2**), obtained as an amorphous powder, $[\alpha]_D$ −26.0° (MeOH), exhibited a molecular formula $C_{42}H_{64}O_{16}$ by HR-FAB-MS. The ^{1}H-NMR spectrum of **2** showed signals due to four *tert*-methyls at δ 0.54, 0.95, 1.45, 1.77, an acetyl at δ 2.14, two anomeric protons at δ 5.03 (1H, d, J=7.9 Hz), 5.06 (1H, d, J=7.9 Hz), an acetoxy-bearing methine proton at δ 5.29 (1H, br s), and an olefinic proton at δ 5.58 (1H, br s). The ^{13}C-NMR spectrum displayed a total of 42 carbon signals as listed in Table 1. They were constituted with three oxygen-bearing methine carbons at δ 73.2, 75.2, 85.2, an oxygen-bearing quaternary carbon at δ 77.0, a tri-substituted double bond at δ 137.6, 124.5, a lactone carbonyl at δ 175.3, an acetyl group at δ 21.2, 170.6, and two anomeric carbon signals at δ 102.3, 105.3. 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. Hence the structure of isotubocaposide B (**2**) was represented as 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl isotubocaposigenin.

Isotubocaposide C (**3**), obtained as an amorphous powder, $[\alpha]_D$ −14.8° (MeOH), gave a molecular formula $C_{42}H_{64}O_{16}$ by HR-FAB-MS. The ^{1}H-NMR spectrum of **3** showed four *tert*-methyls at δ 0.54, 1.10, 1.43, 1.77, an acetyl at δ 1.94, two anomeric protones at δ 5.12 (1H, d, J=7.9 Hz), 5.28 (1H, d, J=7.9 Hz), an acetoxy-bearing methine proton at δ 5.30 (1H, br s), and an olefinic proton at δ 5.53 (1H, br s). The ^{13}C-NMR signals were composed of a total of 42 carbons, which implied three oxygen-bearing methine carbons at δ 74.1, 75.3, 85.1, an oxygen-bearing quaternary carbon at δ 77.0, a tri-substituted double bond at δ 124.2, 137.8, a lactone carbonyl at δ 175.2, an acetyl group at δ 20.9, 170.1, and two anomeric carbons at δ 101.1, 106.7. Isotubocaposide C (**3**) showed a terminal β-D-glucopyranosyl residue and 2-*O*-glycosylated β-D-glucopyranosyl moiety. Therefore the structure of **3** could be represented as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl isotubocaposigenin.

Isotubocaposides have the structural peculiarity of an unusual side chain carrying a C-21 bound to the C-24 on the lactone ring.

Experimental

General Experimental Procedures Column chromatography was carried out with Diaion HP-20 and MCI gel CHP20P (Mitsubishi Kagaku), silica gel 60 and 60N (Kanto Chemical Co., Inc.), 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 by RI detector. TLC was performed on a precoated silica gel 60 F_{254} (Merck) and RP-18 F_{254}S (Merck). Melting points were measured by Yanagimoto micro melting apparatus.

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

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

Extraction and Isolation Air-dried fruits of *T. anomalum* (650 g) were extracted twice with MeOH 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 (0.63 g) of fr. 5 was applied to Chromatorex ODS (200 g) with 50%→53%→55% MeOH to give 581 mg of isotubocaposide A (**1**) together with tubocaposide B (552.3 mg). The residue (1.00 g) of fr. 2 was applied to silica gel (300 g) with $CHCl_3$–MeOH–H_2O=20 : 1 : 0→9 : 1 : 0.1→8 : 2 : 0.2 and fractionated into fr. 2-1—2-14. The residue (50.6 mg) of fr. 2-13 was applied to Chromatorex ODS (25 g) with 60% MeOH and fractionated into fr. 2-13-1—2-13-5. The residue (10.0 mg) of fr. 2-13-4 was purified by HPLC with 55% MeOH to give 3.7 mg of isotubocaposide B (**2**) together with tubocaposide A (1.5 mg). The residue (0.17 g) of fr. 2-11 was applied to Chromatorex ODS (55 g) with 60% MeOH to give 35.4 mg of isotubocaposide C (**3**).

Isotubocaposide A (**1**): An amorphous powder, $[\alpha]_D^{25}$ −26.5° (c=0.1, MeOH). HR-FAB-MS (positive-ion mode) m/z: 1009.45907 $[M+Na]^+$ (Calcd for $C_{48}H_{74}O_{21}Na$ 1009.46203). ^{1}H-NMR (C_5D_5N, 500 MHz) δ 0.53 (3H, s, H_3-18), 1.09 (3H, s, H_3-19), 1.44 (3H, s, H_3-28), 1.77 (3H, s, H_3-27), 2.10 (3H, s, Ac), 4.75 (1H, d, J=10.4 Hz, glc′ H-6), 5.02 (1H, d, J=7.9 Hz, glc″ H-1), 5.06 (1H, d, J=7.3 Hz, glc′ H-1), 5.22 (1H, d, J=7.3 Hz, glc‴ H-1), 5.32 (1H, br s, H-1), 5.57 (1H, br s, H-6), ^{13}C-NMR (C_5D_5N, 500 MHz) δ (Table 1).

Isotubocaposide B (**2**): An amorphous powder, $[\alpha]_D^{25}$ −26.0° (c=0.1, MeOH). HR-FAB-MS (positive-ion mode) m/z: 847.40603 $[M+Na]^+$ (Calcd for $C_{42}H_{64}O_{16}Na$ 847.40920). ^{1}H-NMR (C_5D_5N, 500 MHz) δ 0.54 (3H, s, H_3-18), 0.95 (3H, s, H_3-19), 1.45 (3H, s, H_3-28), 1.77 (3H, s, H_3-27), 2.14 (3H, s, Ac), 5.03 (1H, d, J=7.9 Hz, glc H-1), 5.06 (1H, d, J=7.9 Hz, glc H-1), 5.29 (1H, br s, H-1), 5.58 (1H, br s, H-6), ^{13}C-NMR (C_5D_5N, 500 MHz) δ (Table 1).

Isotubocaposide C (**3**): An amorphous powder, $[\alpha]_D^{25}$ −14.8° (c=0.1, MeOH). HR-FAB-MS (positive-ion mode) m/z: 847.40702 $[M+Na]^+$ (Calcd for $C_{42}H_{64}O_{16}Na$ 847.40920). ^{1}H-NMR (C_5D_5N, 500 MHz) δ 0.54 (3H, s, H_3-18), 1.10 (3H, s, H_3-19), 1.43 (3H, s, H_3-28), 1.77 (3H, s, H_3-27), 1.94 (3H, s, Ac), 5.12 (1H, d, J=7.9 Hz, glc H-1), 5.28 (1H, d, J=7.9 Hz, glc H-1), 5.30 (1H, br s, H-1), 5.53 (1H, br s, H-6), ^{13}C-NMR (C_5D_5N, 500 MHz) δ (Table 1).

Enzymatic Hydrolysis of Isotubocaposide A (1) Isotubocaposide A (**1**) (265 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 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 (377 mg) of the MeOH eluate was applied to silica gel (70 g) with hexane–acetone=4 : 1 to give 70.9 mg of isotubocaposigenin (**4**).

Isotubocaposigenin (**4**): An amorphous powder, $[\alpha]_D^{25}$ −42.2° (c=0.1, $CHCl_3$). HR-FAB-MS (positive-ion mode) m/z: 523.3076 $[M+Na]^+$ (Calcd for $C_{30}H_{44}O_6Na$ 523.3036). ^{1}H-NMR (C_5D_5N, 500 MHz) δ: 0.57 (3H, s, H_3-18), 1.10 (3H, s, H_3-19), 1.44 (3H, s, H_3-28), 1.77 (3H, s, H_3-27), 2.09 (3H, s, Ac), 4.34 (1H, m, H-3), 4.59 (1H, br s, H-22), 5.34 (1H, br s, H-1), 5.59 (1H, br s, H-6), ^{13}C-NMR (C_5D_5N, 500 MHz) δ (Table 1).

Preparation of Isotubocaposigenin *p*-Bromobenzoate (5) A solution of isotubocaposigenin (**4**) (53.2 mg) in pyridine (250 μl) was treated with *p*-bromobenzoyl chloride (203 mg) in CH_2Cl_2 (3 ml) at room temperature for 4 h. The residue of the reaction mixture (243 mg) was applied to silica gel (40 g) with hexane–acetone 10 : 1→9 : 1→8 : 1 to give 54.8 mg of isotubocaposigenin *p*-bromobenzoate (**5**).

Isotubocaposigenin *p*-Bromobenzoate (**5**): Colorless plates, mp 152—154 °C, $[\alpha]_D^{25}$ −31.9° (c=0.1, $CHCl_3$). HR-FAB-MS (positive-ion mode) m/z: 705.24166 $[M+Na]^+$ (Calcd for $C_{37}H_{47}BrO_7Na$ 705.24028). ^{1}H-NMR (C_5D_5N, 500 MHz) δ 0.58 (3H, s, H_3-18), 1.11 (3H, s, H_3-19), 1.44 (3H, s, H_3-28), 1.76 (3H, s, H_3-27), 2.13 (3H, s, Ac), 4.59 (1H, br s, H-22), 5.34 (1H, br s, H-1), 5.46 (1H, m, H-3), 5.58 (1H, br s, H-6), 7.71 (2H, d, J=8.5 Hz), 8.06 (2H, d, J=8.5 Hz), ^{13}C-NMR (C_5D_5N, 500 MHz) δ (Table 1).

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