New Withanolides, Daturataturins A and B from *Datura tatura* L.[1)]

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Two new C_{28} steroidal glycosides called daturataturin A (1a) and daturataturin B (2a) were isolated from the methanolic extract of the fresh aerial parts of *Datura tatura* L. (Solanaceae), and their chemical structures were characterized as (22*R*)-7α,27-dihydroxy-1-oxowitha-2,5,24-trienolide 27-*O*-β-D-glucopyranoside and (22*R*)-1α,3β,7α,-27-tetrahydroxywitha-5,24-dienolide 3-*O*-β-D-glucopyranoside by spectral analysis.

Keywords *Datura tatura*; Solanaceae; withanolide; daturataturin A; daturataturin B

As a part of our studies on the constituents of solanaceous plants, we previously reported the chemical structures of C_{28} steroidal lactones called daturametelins A, B, C, D, E, F and G-Ac from the methanolic extract of the fresh aerial parts of *Datura metel* L.[2)]

In continuing studies on the constituents of the same genus, we report here the isolation and structure elucidation of two new withanolide glucosides, daturataturins A (**1a**) and B (**2a**), from the fresh aerial parts of *D. tatura* L.

Daturataturin A (**1a**) $C_{34}H_{48}O_{10}$, an amorphous powder, showed a $[M+Na]^+$ peak at *m/z* 639, a $[M-H_2O+H]^+$ peak at *m/z* 599 and a $[M-H_2O-glc]^+$ peak at *m/z* 437 in the positive fast atom bombardment mass spectrum (FAB-MS). The infrared (IR) spectrum of **1a** indicated the presence of an α,β-unsaturated δ-lactone (1698 cm^{-1}) and an α,β-unsaturated ketone (1663 cm^{-1}). Acetylation of **1a** with acetic anhydride and pyridine gave a pentaacetate (**1b**), which showed a $[M-AcOH+H]^+$ peak at *m/z* 767 in the positive FAB-MS. By comparison of the proton nuclear magnetic resonance (^{1}H-NMR) spectrum (Table I) of **1b** with that of daturametelin A tetraacetate (**3b**), the following signals in **1b** were assigned: four methyl groups [δ 0.73 (s, H_3-18), 1.04 (d, *J*=6.6 Hz, H_3-21), 1.25 (s, H_3-19) and 2.06 (s, H_3-28)], five acetyl groups [δ 2.00, 2.01, 2.02, 2.03 and 2.09 (all s)], two methylene groups [δ 2.90 (dd, *J*=20.9 and 4.8 Hz, H-4), 3.35 (d, *J*=20.9 Hz, H′-4), 4.47 (d, *J*=11.0 Hz, H-27) and 4.59 (d, *J*=11.0 Hz, H′-27)], three olefinic protons [δ 5.92 (dd, *J*=9.9 and 2.2 Hz, H-2), 6.81 (ddd, *J*=9.9, 5.0 and 2.6 Hz, H-3) and 5.83 (dd, *J*=5.9 and 1.8 Hz, H-6)], and a characteristic H-22 proton [δ 4.41 (dt, *J*=13.2 and 3.3 Hz)]. Furthermore, one methine proton signal (δ 4.90, m) was found to be attributable to H-7 by its correlation with the olefinic proton at H-6 in the ^{1}H–^{1}H correlated spectroscopy (COSY) spectrum. The chemical shift and the coupling pattern due to the H-22 signal indicated the presence of the typical C-17 side chain without a hydroxy group at C-20.[3)] As regards the configuration at C-22, the circular dichroism (CD) spectrum of **1a** showed a positive Cotton effect at 255 nm, suggesting *R* configuration. Moreover, the ^{1}H-NMR spectrum of **1b** revealed the existence of a 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl moiety [δ 4.65 (d, *J*=8.1 Hz, H-1′), 4.94 (dd, *J*=9.6, 8.1 Hz, H-2′), 5.21 (t, *J*=9.6 Hz, H-3′), 5.08 (t, *J*=9.6 Hz, H-4′), 3.69 (ddd, *J*=9.6, 4.4, 2.6 Hz, H-5′), 4.17 (dd, *J*=12.3, 2.6 Hz, H-6′) and 4.23 (dd, *J*=12.3, 4.4 Hz, H′-6′)]. From the evidence of the chemical shifts and coupling patterns of H_3-28 and H_2-27, the location of the glycosidic linkage was determined to be the 27-hydroxy group such as **3b**. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) data for **1a** (Table II) were consistent with the assumed structure. Therefore, the structure of **1a** was concluded to be (22*R*)-7α,27-dihydroxy-1-oxowitha-2,5,24-trienolide 27-*O*-β-D-glucopyranoside. The aglycone moiety of **1a** corresponds to compound IX, which was isolated from *Withania somnifera* DUN. by Kirson *et al.*[4)]

Daturataturin B (**2a**) $C_{34}H_{52}O_{11}$, an amorphous powder, showed a strong absorption band at 3412 cm^{-1} (hydroxyl) and a characteristic band at 1694 cm^{-1} (α,β-unsaturated

TABLE I. ^{1}H-NMR Data ($CDCl_3$) for Daturataturin A Pentaacetate (**1b**) and Daturataturin B Heptaacetate (**2b**)

Proton	1b	2b
1	—	5.07 (br s)
2	5.92 (dd) *J*=9.9, 2.2 Hz	
3	6.81 (ddd) *J*=9.9, 5.0, 2.6 Hz	3.88 (m)
4	2.90 (dd) *J*=20.9, 4.8 Hz 3.35 (d) *J*=20.9 Hz	
6	5.83 (dd) *J*=5.9, 1.8 Hz	5.77 (d) *J*=4.0 Hz
7	4.90 (m)[a)]	4.92 (m)[b)]
18	0.73 (s)	0.70 (s)
19	1.25 (s)	1.26 (s)
21	1.04 (d) *J*=6.6 Hz	1.03 (d) *J*=6.6 Hz
22	4.41 (dt) *J*=13.2, 3.3 Hz	4.41 (br d) *J*=9.9 Hz
27	4.47 (d) *J*=11.0 Hz 4.59 (d) *J*=11.0 Hz	4.91 (d) *J*=11.5 Hz 4.86 (d) *J*=11.5 Hz
28	2.06 (s)	2.05 (s)[c)]
Glc-1′	4.65 (d) *J*=8.1 Hz	4.58 (d) *J*=8.0 Hz
Glc-2′	4.94 (dd)[a)] *J*=9.6, 8.1 Hz	4.94 (dd)[b)] *J*=9.6, 8.0 Hz
Glc-3′	5.21 (t) *J*=9.6 Hz	5.18 (t) *J*=9.6 Hz
Glc-4′	5.08 (t) *J*=9.6 Hz	5.05 (t)[b)] *J*=9.6 Hz
Glc-5′	3.69 (ddd) *J*=9.6, 4.4, 2.6 Hz	3.68 (ddd) *J*=9.6, 5.0, 2.5 Hz
Glc-6′	4.17 (dd) *J*=12.3, 2.6 Hz 4.24 (dd) *J*=12.3, 4.4 Hz	4.10 (dd) *J*=12.3, 2.5 Hz 4.23 (dd) *J*=12.3, 5.0 Hz
–OAc	2.00, 2.01, 2.02, 2.03, 2.09	2.00,[c)] 2.02, 2.04, 2.05, 2.08

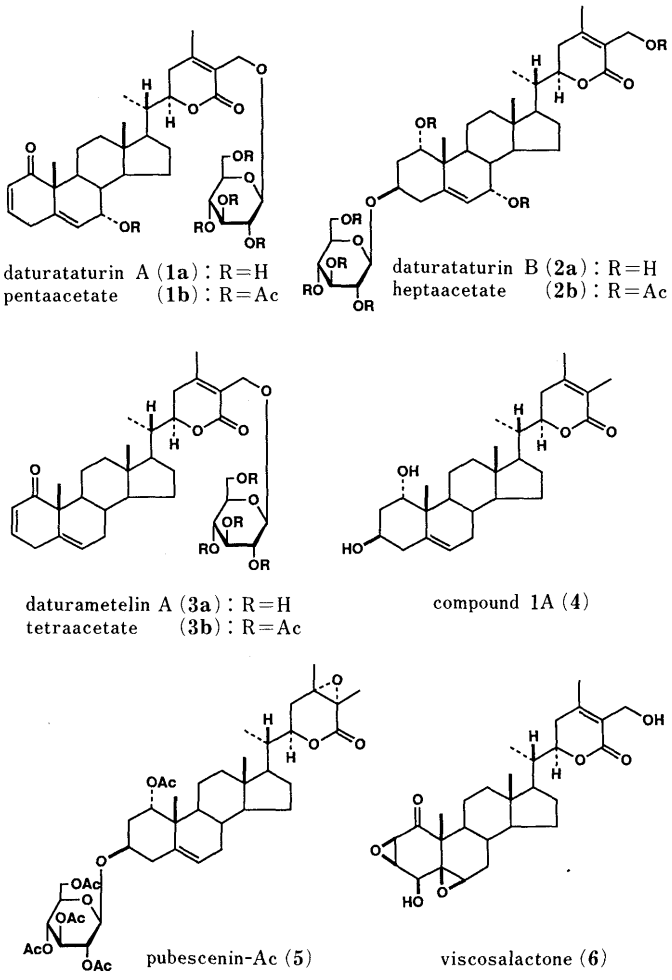
a—c) Signals overlap each other in each vertical column.

TABLE II. ^{13}C-NMR Data for Daturataturin A (**1a**),[a] Daturataturin B (**2a**),[a] Daturametelin A (**3a**),[a] Compound 1A (**4**),[b] Pubescenin Ac (**5**)[b] and Viscosalactone (**6**)[b]

	1a	2a	3a	4	5	6
C-1	203.3	72.3	203.9	72.9	74.9	206.9
C-2	127.9	37.6	127.7	38.3	33.7	54.9
C-3	145.6	73.8	145.8	66.3	74.9	55.8
C-4	33.6	38.2	33.4	41.4	38.1	74.6
C-5	139.4	142.7	136.2	137.6	136.7	64.0
C-6	128.1	127.8	124.6	125.2	124.9	59.6
C-7	63.7	64.7	30.8	31.7	28.8	29.6
C-8	35.8	34.0	33.1	31.3	31.7	31.0
C-9	39.1	39.2	43.3	41.5	42.3	42.7
C-10	50.2	42.9	50.6	41.7	40.5	48.6
C-11	23.9	20.4	23.8	20.2	20.7	20.0
C-12	39.9	39.4	39.8	39.8	39.6	27.3
C-13	42.5	42.7	42.5	43.0	42.8	42.7
C-14	52.2	52.2	56.2	56.8	56.5	56.1
C-15	24.3	24.6	24.2	23.9	24.3	24.3
C-16	27.2	27.4	26.9	22.0	27.3	40.7
C-17	51.3	49.7	51.9	54.7	52.1	51.9
C-18	11.9	11.7	11.7	13.6	11.7	11.5
C-19	18.2	18.3	18.8	19.4	19.4	14.5
C-20	38.7	38.9	38.9	75.2	38.6	38.9
C-21	13.4	13.5	13.3	20.8	13.7	13.3
C-22	78.3	78.2	78.2	81.0	76.3	78.7
C-23	29.8	29.8	29.9	31.5	31.7	29.8
C-24	157.1	153.9	157.0	149.1	59.3	153.8
C-25	In solvent	127.3	122.8	122.0	62.6	125.8
C-26	166.1	166.4	166.0	166.2	170.6	167.4
C-27	63.4	56.2	63.2	12.5	13.0	57.2
C-28	20.6	20.1	20.4	20.5	18.0	20.1
Glc-1′	104.8	102.9	104.6		99.6	
Glc-2′	75.2	75.2	75.0		71.4	
Glc-3′	78.5	78.5	78.3		72.9	
Glc-4′	71.6	71.5	71.5		68.5	
Glc-5′	78.4	78.4	78.3		71.8	
Glc-6′	62.8	62.7	62.6		62.1	
–OAc					170.3	
					170.0	
					169.4 (×2)	
					20.7 (×4)	
					21.1	

a) In pyridine-d_5, b) in $CDCl_3$.

δ-lactone) in its IR spectrum. The positive FAB-MS of **2a** showed a $[M+Na]^+$ peak at *m/z* 659. Next, the ^{1}H- and ^{13}C-NMR spectral data (Tables I and II, respectively) of **2a** and its heptaacetate (**2b**) were compared with those of compound 1A (**4**)[5] and pubescenin-Ac (**5**)[6] (for the A, B-ring moiety), and viscosalactone (**6**)[7] (for the side chain). The ^{1}H-NMR spectrum (Table I) of **2b** disclosed the occurrence of four methyl groups [δ 0.70 (H_3-18), 1.03 (H_3-21), 1.26 (H_3-19) and 2.05 (H_3-28)], one oxymethyl group [δ 4.91 and 4.86 (H_2-27)] and two oxygenated methine protons [δ 3.88 (H-3) and 4.41 (H-22)] together with the presence of the β-D-glucopyranosyl moiety. The chemical shifts of this oxymethyl signal in **2b** indicated that the glucosyl moiety did not link to the C-27 hydroxymethyl group, in contrast to **1b**. Furthermore, in the ^{1}H-NMR spectrum of **2b**, the olefinic protons at C-2 and C-3 in ring A of **1b** disappeared, and instead, two new oxygenated methine proton signals occurred at δ 3.88 (1H, m) and 5.07 (1H, br s). Thus, the steroidal 2-ene-1-one system in **1a** no longer existed in **2a**. These new methine protons were assigned to the 5-ene-1α,3β-diol system, which is known as



the precursor of the 2-ene-1-one system in the biogenesis of the withanolides.[3] The ^{13}C-NMR data of the side chain moiety (Table II) of the side chain part for **2a** were in good accordance with those of **6** except for C-25. Further, by comparison of the ^{13}C-NMR data of **2a** with those of **4** and **5**, the presence of a 5-ene-1α,3β-diol structure (*vide supra*) and the β-D-glucopyranosyl group linked to the 3β-hydroxy group in **2a** (and also **2b**) were reasonably clarified. Based on these spectral data and from the positive Cotton effect at 252 nm, the structure of **2a** having the (22*R*)-configuration was shown in the formula.

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter and CD spectra on a JASCO J-50A spectropolarimeter. The IR spectra were recorded with a Hitachi IR spectrometer, model 270-30. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 NMR spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. The FAB-MS were taken in a glycerol matrix containing NaI. Thin layer chromatography was performed on precoated Kieselgel 60 F_{254} (Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (70—230 mesh and 230—400 mesh, Merck) and Sephadex LH-20 (Pharmacia Co.).

Extraction and Separation Frash aerial parts (1.78 kg) of *Datura tatura* L. (Solanaceae) harvested at the botanical garden in Kumamoto University in July 1988 were extracted with MeOH and the extract (40.3 g) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer (16.3 g) was subjected to column chromatography repeatedly over silica gel using $CHCl_3$–MeOH–H_2O = 1 : 0 : 1 → 8 : 2 : 0.1 → 0 : 1 : 0 and over Sephadex LH-20 using MeOH to give daturataturins A (**1a**, 1.70 g) and B (**2a**, 20.7 mg).

Daturataturin A (1a) An amorphous powder, $[\alpha]_D^{21}$ −38.1° (c=0.72, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3442, 1698, 1663, 1464, 1404, 1344, 1323. Positive FAB-MS m/z: 639 $[M+Na]^+$, 599 $[M-H_2O+H]^+$, 437 $[M-glc-H_2O+H]^+$, 419 $[M-glc-2H_2O+H]^+$, 401 $[M-glc-3H_2O+H]^+$, 265, 171. CD (c=0.94, MeOH) $[\theta]$ (nm): −25000 (325) (negative max.), +47000 (255) (positive max.).

Acetylation of Daturataturin A (1a) A solution of **1a** (109 mg) in pyridine–acetic anhydride (1 : 1, v/v, 4 ml) was kept at room temperature overnight and the product was chromatographed on silica gel (n-hexane–EtOAc = 1 : 1, v/v) to afford a pentaacetate (**1b**, 53.4 mg). An amorphous powder, $[\alpha]_D^{33}$ −42.0° (c=0.59, $CHCl_3$). Positive FAB-MS m/z: 767 $[M-AcOH+H]^+$, 331.

Daturataturin B (2a) An amorphous powder, $[\alpha]_D^{15}$ −17.7° (c=0.52, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3412, 1694, 1466, 1400, 1386, 1320, 1306. Positive FAB-MS m/z: 659 $[M+Na]^+$, 619 $[M-H_2O+H]^+$, 439 $[M-glc-2H_2O+H]^+$, 289, 232, 176. CD (c=0.60, MeOH) $[\theta]$ (nm): −4770 (315) (negative max.), +48700 (252) (positive max.).

Acetylation of Daturataturin B (2a) Acetylation was performed with pyridine and acetic anhydride (2 : 1, v/v, 3 ml) overnight at room temperature. The product was purified on silica gel column chromatography (benzene–acetone = 20 : 1, v/v), and provided a heptaacetate (**2b**, 11.1 mg). An amorphous powder, $[\alpha]_D^{25}$ −31.9° (c=0.53, $CHCl_3$). Positive FAB-MS m/z: 953 $[M+Na]^+$, 871 $[M-AcOH+H]^+$, 811 $[M-2AcOH+H]^+$, 601 $[M-glc(Ac)+H]^+$, 550, 522, 331, 289, 246.

References and Notes

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