TWO NEW ERGOSTANE DERIVATIVES FROM TUBOCAPSICUM ANOMALUM (SOLANACEAE)[1)]

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Two new steroidal sapogenols were obtained from the hydrolysate of the methanolic extract of the fresh aerial parts of Tubocapsicum anomalum Makino. They were found by various spectral and X-ray analyses to be ergostane derivatives having novel structures as if the lactone ring of withanolide opened and the 18-methyl group shifted to C-17.

KEYWORDS Tubocapsicum anomalum; Solanaceae; ergostane derivative; withanolide; X-ray analysis

As a part of studies on the withanolides in solanaceous plants, we recently isolated two new C_{28} steroidal lactone glycosides named tubocaposides A and B from the fresh berries of Tubocapsicum anomalum Makino and determined their structures by X-ray analysis.[2)] Tubocaposides have a novel side chain with a C-21 methyl group attached to the C-24 on the lactone ring, and a 3-O-glycosidic linkage at the saturated A-ring. Continuing research on this plant led to the isolation of two more novel steroidal sapogenols from the hydrolysate of a methanolic extract of the fresh aerial parts. Here we describe their chemical structures.

The methanololic extract (31.5 g) of the fresh aerial parts (571 g) of Tubocapsicum anomalum was hydrolyzed with 2N HCl-MeOH, then neutralized. Then the methanolysate was partitioned between EtOAc and water. Silica gel column chromatography of the EtOAc layer (18 g) (n-hexane-EtOAc and $CHCl_3$-MeOH) was used to isolate TH-6 (26.6 mg) and TH-12 (35.2 mg).

TH-6, colorless needles, mp 190-192.1°C, $[\alpha]_D$ -120.4°($CHCl_3$), positive FAB-MS (m/z): 519 $[M+H]^+$, exhibited absorption bands at 3520 cm^{-1} (hydroxyl), 1698 cm^{-1} (α,β-unsaturated carboxyl) and 1676 cm^{-1} (α,β-unsaturated carbonyl) in its IR spectrum. The ^{1}H-NMR spectrum ($CDCl_3$) of TH-6 showed signals due to a sec-methyl [δ 0.79 (3H, d, J=7.0 Hz)], two steroidal angular methyls [δ 1.10, 1.23 (each 3H, s)], two vinyl methyls [δ 1.88, 1.89 (each 3H, s)], one ester methyl [δ 3.70 (3H, s)], four protons [δ 4.10 (2H, m), 4.48 (1H, dd, J=4.8, 12.8 Hz), 5.07 (1H, br s)] geminal to the electron drawing function groups and two cis olefinic protons [δ 6.05 (1H, dd, J=2.2, 10.6 Hz), 6.53 (1H, dd, J=2.4, 10.6 Hz)]. The ^{13}C-NMR spectrum (Table I) of TH-6 showed it to be an ergostane derivative with a 1-one-2-en-4,5-dihydroxy structure [δ 200.2 (s, C-1), 127.6 (d, C-2), 143.2 (d, C-3), 66.9 (d, C-4), 78.2 (s, C-5)] in the A-ring by comparing it with withapervin B.[3)] We also found the side chain moiety of a 2-carbomethoxy-3-methyl-2-butenyl group [δ 146.2 (s, C-24), 123.2 (s, C-25), 169.7 (s, C-26), 10.9 (q, Me-27), 21.7 (q, Me-28)].

To determine the obscure structure of TH-6, especially with respect to the location of the two sp^2 carbons [δ 137.8 (s), 134.6 (s)] and three carbons [δ 66.5 (d), 82.6 (d), 88.9 (d)] attached to the electron drawing atoms, we conducted a single crystal X-ray

diffraction study of the substance.

The crystal data were orthorhombic, space group $P2_12_12$, a=1.004(2), b=20.677(4), c=12.153(2) Å, V=2765.3 Å^3, Z=4. All data were collected on an Enraf-Nonius CAD4F-11 diffractometer using Cu-Kα radiation and a graphite monochrometer. The X-ray crystal structure of TH-6 is illustrated as a quite novel natural model in Fig. 1.

TH-12, a white powder, $[\alpha]_D$ -65.8°($CHCl_3$), positive FAB-MS (m/z): 523 $[M+Na]^+$, 501 $[M+H]^+$, showed absorption bands at 3448 cm^{-1} (hydroxyl), 1712 cm^{-1} (α,β-unsaturated carboxyl) and 1682 cm^{-1} (α,β-unsaturated carbonyl) in its IR spectrum. A comparison of the ^1H- and ^{13}C-NMR spectra of TH-12 with those of TH-6 enabled us to assign the respective signals as follows: six methyl groups [δ 0.78 (3H, d, J=7.3, 21-Me), 1.11 (3H, s, 18-Me), 1.16 (3H, s, 19-Me), 1.87 (3H, s, 27-Me), 1.88 (3H, s, 28-Me), 3.70 (3H, s, C-26 ester

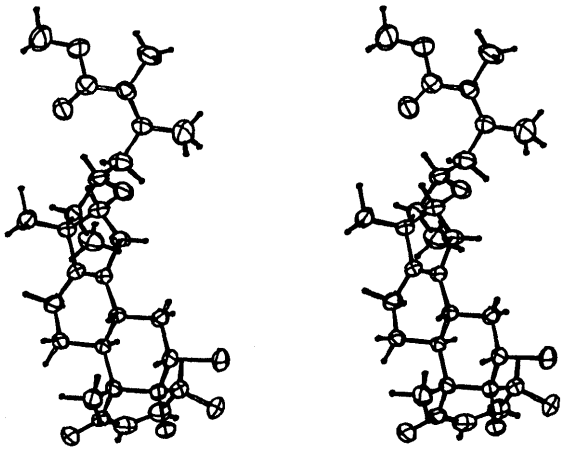
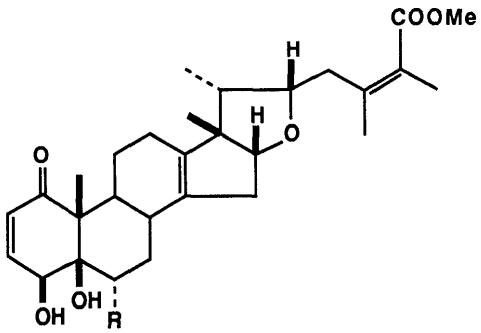


Fig. 1. ORTEP Drawing of TH-6



TH-6 R = Cl
TH-12 R = OH

Table I. ^{13}C-NMR Data for TH-6 and TH-12 (in $CDCl_3$)

	TH-6	TH-12		TH-6	TH-12
C-1	200.2	201.1	C-16	82.6	82.8
2	127.6	127.7	17	60.6	60.7
3	143.2	144.7	18	23.8	23.8
4	66.9[a)]	67.0	19	10.0	10.3
5	78.2	78.9	20	43.4	42.5
6	66.5[a)]	75.2	21	15.9	16.0
7	38.5	35.9	22	88.9	89.0
8	36.1	35.0	23	39.1	39.2
9	46.1	46.1	24	146.7	146.7
10	57.1	55.6	25	123.2	123.3
11	24.1[b)]	24.1	26	169.7	169.8
12	24.3[b)]	24.5	27	10.9	10.8
13	134.6	135.4	28	21.7	21.9
14	137.8	137.4	COOMe	51.3	51.3
15	37.3	37.4			

a, b) Assignments may be interchangeable within the same column.

methyl)], four protons [δ 4.34 (1H, br s, H-16), 5.12 (1H, br s, H-4), 4.08 (2H, m, H-6 and H-22)] geminal to the hydroxyl groups, two olefinic protons [δ 6.01 (1H, d, J=10.4 Hz, H-2), 6.56 (1H, d, J=10.3 Hz, H-3)]. The ^{13}C-NMR assignments for TH-12 are listed in Table I. The chlorine group at C-6 on the B-ring in TH-6 was found to be introduced instead of the hydroxy group in TH-12 by comparing the ^{1}H- and ^{13}C-NMR spectra of TH-6, TH-12 and TH-12 diacetate[4] with those of withapervin diacetate[5] and withaperuvin B.[3] Thus, the structure of TH-12 was determined as shown in the formula.

Even though TH-6 and TH-12 may be artificially formed during acid hydrolysis from the corresponding typical withanolide (Chart 1), they are worthy of note with regard to their structural novelties including an interesting mechanism for methyl migration.

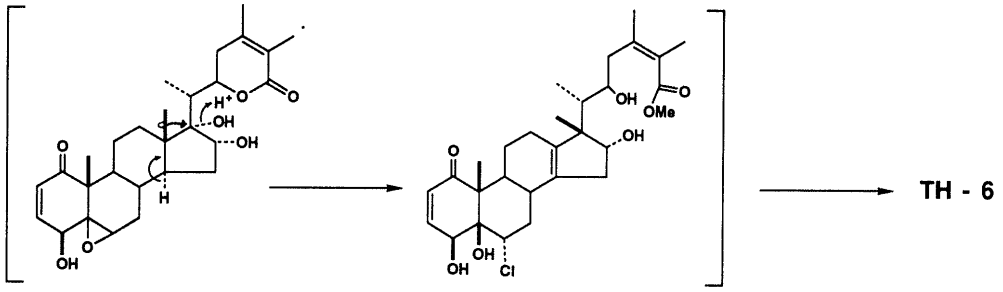


Chart 1

ACKNOWLEDGEMENT We are grateful to Mr. S. Setoguchi, Research Laboratories, Yoshitomi Pharmaceutical Industries Ltd., for facilities for X-ray measurement.

REFERENCES AND NOTES

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4) ^{1}H-NMR data for TH-12 diacetate (in $CDCl_3$) δ : 6.37 (1H, dd, J=10.6, 2.2 Hz, H-3), 6.35 (1H, br s, H-4), 6.11 (1H, dd, J=10.6, 2.9 Hz, H-2), 5.14 (1H, dd, J=12.1, 4.8 Hz, H-6), 4.10 (1H, ddd, J=12.1, 5.3, 2.7 Hz, H-22), 4.05 (1H, dd, J=7.1, 2.4 Hz, H-16), 3.70 (3H, s, COOMe), 2.19, 2.05(each 3H, s, 2xOAc), 1.92, 1.88(each 3H, s, 27-Me, 28-Me), 1.26 (3H, s, 19-Me), 1.10 (3H, s, 18-Me), 0.81 (3H, d, J=7.0 Hz, 21-Me).

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(Received January 16, 1990)