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Phytochemical Studies on Meliaceous Plants. IV.[1] Structure of a New Pregnane Glycoside, Toosendanoside, from Leaves of *Melia toosendan* SIEB. *et* ZUCC.[2]

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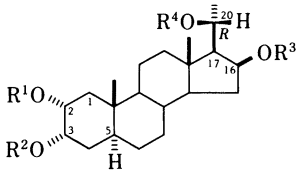
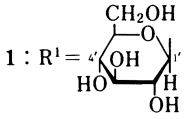
A new pregnane glycoside, named toosendanoside (**1**), has been isolated from leaves of *Melia toosendan* SIEB. *et* ZUCC. (Meliaceae). The structure of **1** has been assigned as (20*R*)-5α-pregnane-2α,3α,16β,20-tetrol 2-*O*-β-D-glucopyranoside, based on lines of chemical and spectral evidence.

Keywords——*Melia toosendan*; Meliaceae; leaf; pregnane glycoside; polyoxypregnane glucoside; toosendanoside

A Chinese crude drug "Lian-ye" (Ren-yoh in Japanese), leaves of *Melia toosendan* SIEB. *et* ZUCC.[3] [*M. azedarach* L. var. *toosendan* (SIEB. *et* ZUCC.) MAKINO[4]] (Meliaceae) has so far been used in China as an anodyne for malaria, uredo, sting, stomach-ache due to roundworms, *etc.*,[3] and as an insecticide.[3,4b] As a part of our phytochemical studies on meliaceous plants, we have recently identified two new pregnane steroids from leaves of *M. toosendan*.[1]

In our continuing phytochemical research on the same material, a new pregnane glycoside, named toosendanoside (**1**), was isolated, after chromatographic and high-pressure liquid chromatographic (HPLC) separation of the *n*-butanol fraction of the methanol extracts, and the whole structure was elucidated on the basis of chemical and spectral evidence.





1: R^1 = 4'-glucopyranosyl, $R^2=R^3=R^4=H$

2: $R^1=R^2=R^3=R^4=H$

3: $R^1, R^2 = >C(CH_3)_2$, $R^3, R^4 = >C(CH_3)_2$

Chart 1

Toosendanoside (**1**) possessed the molecular formula $C_{27}H_{46}O_9$, based on the molecular (M^+) peak at *m/z* 514 in the field desorption mass spectrum (FD-MS) and the $M^- - H$ peak at *m/z* 513 in the negative ion fast atom bombardment mass spectrum (FAB-MS). The electron impact mass spectrum (EI-MS) gave the base peak at *m/z* 299 ($M^+ + H - 162 - 3H_2O$), which arises from the M^+ ion by the loss of a hexose (162) unit and three molecules of water. In addition to this, the proton nuclear magnetic resonance (^{1}H-NMR) (Table II)[5] and carbon-13 nuclear magnetic resonance (^{13}C-NMR) (Table III)[6] data were indicative of the presence of a glucopyranose moiety in **1**. Furthermore, the ^{1}H-NMR data (Table I) of **1** showed signals due to two tertiary methyls [δ 0.78 (19-H_3) and 1.41 (18-H_3)], a secondary methyl [δ 1.73 (d,

TABLE I. ^{1}H-NMR (400 MHz) Data[a] for Aglycone Part of **1**, **2**, and **3**

	1[b]	**2**[b]	**3**[c]
1α-H	1.8[d]	1.8[d]	1.00, dd, 1α,1β = 12.8; 1α,2β = 10.7
1β-H	1.95, dd, 1β,1α = 11.9; 1β,2β = 4.6	1.99, dd, 1β,1α = 12.5; 1β,2β = 4.3	1.94, dd, 1β,1α = 12.8; 1β,2β = 6.6
2β-H	4.08, m (d-like)	4.07, m[f]	4.11, ddd, 2β,1α = 10.7; 2β,1β = 6.6; 2β,3β = 4.0
3β-H	4.5[d]	4.35, m (s-like)	4.19, dt, 3β,4α = 1.2; 3β,2β = 3β,4β = 4.0
4α-H	1.8[d]	1.82, dt, 4α,4β = 14.0; 4α,3β = 4α,5α = 3.4	1.87, ddd, 4α,4β = 15.4; 4α,3β = 1.2; 4α,5α = 4.0
4β-H	1.5[d]	1.6[d]	1.62, ddd, 4β,4α = 15.4; 4β,3β = 4.0; 4β,5α = 13.0
5α-H	1.90[e]	1.97, tt, 5α,4α = 5α,6α = 3.4; 5α,4β = 5α,6β = 12.5	1.5[d]
14α-H	0.9[d]	1.0[d]	0.89, m
15α-H	2.31, dt, 15α,15β = 12.8; 15α,14α = 15α,16α = 7.6	2.34, ddd, 15α,15β = 13.1; 15α, 14α = 7.3; 15α,16α = 7.6	2.14,[h] dt, 15α,15β = 13.4; 15α,14α = 15α,16α = 7.6
15β-H	1.6[d]	1.5[d]	1.27,[h] ddd, 15β,15α = 13.4; 15β, 14α = 13.1; 15β,16α = 3.1
16α-H	4.5[d]	4.55, td, 16α,15α = 16α,17α = 7.6; 16α,15β = 4.6	4.41, ddd, 16α,15α = 7.6; 16α,15β = 3.1; 16α,17α = 5.8
17α-H	1.5[d]	1.53, dd, 17α,16α = 7.6; 17α,20 = 10.1	0.94, dd, 17α,16α = 5.8; 17α,20 = 4.4
20-H	4.76, m	4.78, m[g]	4.36, dq, 20,17α = 4.4; 20,21 = 6.9
18-H_3	1.41, s	1.44, s	1.08, s
19-H_3	0.78, s	0.88, s	0.73, s
21-H_3	1.73, d, 21,20 = 5.8	1.75, d, 21,20 = 5.8	1.34, d, 21,20 = 6.9
Other CH_3			1.31, s; 1.33, s; 1.44, s; 1.50, s

a) Chemical shifts are in δ (ppm) relative to internal TMS, and are followed by multiplicities and coupling constants (Hz). *b*) In pyridine-d_5. *c*) In $CDCl_3$. *d*) Multiplicities and signal patterns were unclear, due to partial overlap, but the assignments for these proton signals were verified based on ^{1}H–^{1}H COSY experiments. *e*) Becomes tt (5α,4α = 5α,6α = 2.4, 5α,4β = 5α,6β = 12.5 Hz) after D_2O exchange. *f*) Becomes ddd (2β,1α = 11.0, 2β,1β = 4.3, 2β,3β = 3.4 Hz) after D_2O exchange. *g*) Becomes dq (20,17α = 10.1, 20,21 = 5.8 Hz) after D_2O exchange. *h*) The present signal assignments for 15α-H and 15β-H are reversed from the previous assignments given in ref. 9.

J = 5.8 Hz)(21-H_3)], and four secondary carbinyl protons, suggesting the presence of a pregnane steroid bearing four secondary hydroxyls as the aglycone part of **1**.

On enzymic hydrolysis with Molsin (protease type XIII from *Aspergillus saitoi*),[7] the glycoside **1** afforded the corresponding genuine aglycone (**2**) $C_{21}H_{36}O_4$ (based on the FD- and accurate MS data), mp 281—283 °C, $[\alpha]_D$ +22.0 °. The detailed ^{1}H-NMR assignments (Table I) were made with the aid of ^{1}H–^{1}H correlated spectroscopy (COSY), and thus, the established data suggested the presence of 2α,3α-glycol, 5α-H (*trans* A/B junction), 16β-OH, 17α-H, and 20-OH units in pregnane **2**, *i.e.*, disclosed that **2** is 5α-pregnane-2α,3α,16β,20-tetrol. The 2α,3α-glycol structure in the 5α-steroid (**2**) was also confirmed by the following ^{13}C-NMR study. The chemical shift for each carbon on ring A in **2** (Table III) was in agreement with that published for 5α-spirostane-2α,3α-diol,[8] and differed from those reported for the corresponding 2β,3α- and 2α,3β-diols.[8] (20*R*)-5α-Pregnane-2α,3α,16β,20-tetrol has already been reported as an alkaline hydrolysis product of natural azedarachol.[9] However, only the melting point (mp 280—282 °C) and M^+ + H ion (*m/z* 353 in FD-MS) were given as the physical and spectral data for this known pregnane.[9] Therefore, as described above, we independently elucidated the structure of **2** based on the ^{1}H-NMR (Table I) and ^{13}C-NMR (Table III) data for **2**.

TABLE II. ^{1}H-NMR (400 MHz) Data[a] for the Sugar Part of **1**, δ (ppm) from TMS

Proton	Sugar part of **1**	Proton	Sugar part of **1**
1′-H	5.07 (d, 7.9)	4′-H	4.36 (dd, 9.5, 8.9)
2′-H	4.11 (dd, 7.9, 9.2)	5′-H	4.05[b]
3′-H	4.21 (dd, 9.2, 9.5)	6′-H_2	4.32 (dd, 11.9, 5.5)
			4.55[b]

a) Measured in pyridine-d_5 after treatment with D_2O. Multiplicities and *J*-values (Hz) in parentheses. *b*) Multiplicities and signal patterns were unclear, due to partial overlap. However, assignments for these protons were established based on ^{1}H–^{1}H COSY.

TABLE III. ^{13}C-NMR (100.5 MHz) Data[a] for **1** and **2**, δ (ppm) from TMS in Pyridine-d_5

	1	**2**		**1**	**2**
C-1	38.66 (t)	41.97 (t)	C-15	38.66 (t)	38.68 (t)
C-2	78.40 (d)	69.13 (d)	C-16	71.82 (d)	71.85 (d)
C-3	68.40 (d)	69.88 (d)	C-17	64.21 (d)	64.21 (d)
C-4	34.89 (t)	35.66 (t)	C-18	14.37 (q)	14.45 (q)
C-5	38.86 (d)	38.88 (d)	C-19	12.64 (q)	12.86 (q)
C-6	28.20 (t)	28.40 (t)	C-20	66.50 (d)	66.51 (d)
C-7	32.52 (t)	32.68 (t)	C-21	24.73 (q)	24.76 (q)
C-8	34.75 (d)	34.82 (d)	C-1′	103.99 (d)	
C-9	55.01 (d)	55.16 (d)	C-2′	75.31 (d)	
C-10	37.22 (s)	37.31 (s)	C-3′	79.30 (d)	
C-11	21.12 (t)	21.16 (t)	C-4′	71.76 (d)	
C-12	40.82 (t)	40.93 (t)	C-5′	78.55 (d)	
C-13	43.29 (s)	43.34 (s)	C-6′	62.82 (t)	
C-14	54.33 (d)	54.41 (d)			

a) Multiplicities (in parentheses) were determined by INEPT experiments. Assignments for both compounds were made with the aid of the ^{13}C–^{1}H COSY method.

The stereochemistry at C-20 in **2** was inferred in a similar manner to that mentioned in ref. 9. A diacetonide (**3**) (mp 174—176 °C) of **2** had the same melting point and molecular ion (M^+, *m/z* 432 in FD-MS) as those reported for a known diacetonide (mp 182—184 °C) derived from azedarachol.[9] In the ^{1}H-NMR spectrum of **3**, principal protons in **3** (Table I) were reasonably assigned with the aid of ^{1}H–^{1}H COSY and double resonance experiments. The chemical shifts and *J*-values of these protons were essentially consistent with those published for the known diacetonide,[9] although a discrepancy between the chemical shifts for 1α-H (**3**, δ 1.00[10]; ref. 9, δ 1.60) was apparent. Thus, it may be concluded that the present diacetonide (**3**) has the same structure as the reported one.[9] The configuration at C-20 in **3** (also in **2**) was inferred to be *R* by detailed analyses of the ^{1}H-NMR data of **3** (Table I) in the same manner as in ref. 9. Inspection of a Dreiding model of **3** revealed that the dihedral angles between H-20 and H-17α are, respectively, *ca.* 20° and *ca.* 90° for α- and β-orientations of the H-20, assuming the six-membered ring containing C-20 to be in the most probable chair form.[11] The observed *J*-value (4.4 Hz) between H-20 and H-17α was consistent with the *ca.* 20° dihedral angle, which suggests the C-20 configuration in **3** to be α (=20*R*). The accumulated evidence led us to (20*R*)-5α-pregnane-2α,3α,16β,20-tetrol as the structure for **2**.

On methanolysis, the glycoside **1** afforded methyl glucoside. Furthermore, the difference in molecular rotation between **1** and **2** ($\Delta[M]_D$ −53.18°) indicated that the glucose moiety in **1** is in a form of β-D-glucopyranose.[12] The β-glucopyranosyl (C1 conformation) moiety in **1** was also corroborated by ^{1}H-NMR [the anomeric proton signal with a large coupling

constant ($J=7.9$ Hz) and the other proton signals due to the glucose] (Table II) and ^{13}C-NMR data (giving chemical shifts consistent with glucopyranosyl carbons) (Table III).

The whole structure for **1** was established as follows. The position (on the aglycone) where the glucosyl group is connected was decided by examining the glycosylation shifts between the glycoside **1** and aglycone **2** in the ^{13}C-NMR spectra. Atom C-2 of **1** resonated at δ78.40 ppm downfield (by 9.3 ppm) from the corresponding signal (δ 69.13) for **2**, while in contrast, the C-1 and C-3 signals (δ 38.66 and 68.40 ppm, respectively) of **1** appeared upfield (by 1.5—3.3 ppm) from those (δ 41.97 and 69.88 ppm, respectively) of **2**. These lines of spectral evidence suggest that in **1**, the glucosyl moiety is linked with the 2α-OH group on the pregnane steroid (**2**) through a glycosidic linkage.

Based on the combined evidence, the structure for **1** is defined as (20*R*)-5α-pregnane-2α,3α,16β,20-tetrol 2-*O*-β-D-glucopyranoside.

Experimental

The instruments used to obtain melting points, infrared (IR), ^{1}H-NMR (400 MHz), ^{13}C-NMR (100.5 MHz), and MS data, and optical rotations, are the same as described in our preceding paper.[1] Melting points are uncorrected. Unless otherwise mentioned, ^{1}H- and ^{13}C-NMR spectra were measured with pyridine-d_5 as a solvent and with tetramethylsilane (TMS) as an internal standard. MS data were obtained under the following conditions (EI-MS and accurate MS: ionization voltage, 30 eV. FD-MS: carbon emitter; accelerating voltage, 3 kV; emitter current, 5—22 mA; chamber at room temperature. Negative ion FAB-MS: accelerating voltage, 2—3 kV; matrix, triethanolamine; collision gas, Xe). Optical rotations were determined for solutions in MeOH. Gas liquid chromatography (GLC) was carried out on a Shimadzu GC-7AG gas chromatograph under the following operating conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m × 3 mm i.d.); FID detector; column temperature, 182 °C; carrier N_2 gas, 32 ml/min. For column chromatography, Merck Kieselgel 60 (230—400 mesh) and Sephadex LH-20 were used and for thin layer chromatography (TLC) and high-performance TLC (HPTLC), precoated silica gel plates, Merck HF-254 and Si50000F-254S, respectively. Preparative HPLC was performed on a Kusano instrument with a KPW-10 micro-pump and a Shodex SE-31 differential refractometer. In HPLC separation, a reversed-phase Kusano ODS column [10 cm × 22 mm i.d.; mobile phase, MeOH–H_2O (1 : 1); flow-rate, 3 ml/min] and a Kusano Si-10 silica column [10 cm × 22 mm i.d.; mobile phase, $CHCl_3$–MeOH (6 : 1); flow-rate, 3 ml/min] were used in that order. Molsin (protease type XIII from *Aspergillus saitoi*) was a commercial product (Sigma Chem. Co., Lot. No. 104F-0124).

Plant Material——The same as mentioned in ref. 1.

Isolation of Toosendanoside (1)——The air-dried leaves (1.5 kg) were extracted twice with MeOH (20 l) at room temperature for a week, and the solvent was evaporated off under reduced pressure. The combined extract (287 g) was suspended in H_2O and the aqueous suspension was extracted successively with petroleum ether (500 ml × 3), $CHCl_3$ (500 ml × 4), and *n*-BuOH (400 ml × 2). The residue (52.4 g) obtained from the *n*-BuOH layer was subjected to column chromatography [silica gel, 1 kg; eluent, $CHCl_3$–MeOH–H_2O (35 : 20 : 4)] and a fraction (2.87 g) containing **1** was separated. This fraction was further purified by HPLC separation to give pure toosendanoside (**1**), colorless needles of mp 265.5—268.5 °C (Me_2CO), $[\alpha]_D$ −8.1° ($c=0.21$). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2900, 1070, 1030. FD-MS *m/z* (%): 515 (M^++H, 35), 514 (M^+, 8), 513 (M^+ − H, 27), 496 ($M^+ - H_2O$, 23), 469 [M^+ − side chain (C_2H_5O), 7], 451 [$M^+ - H_2O$ − side chain (C_2H_5O), 6], 335 ($M^+ + H - 162 - H_2O$, 13), 179 (100). Negative ion FAB-MS *m/z* (%): 513 (M^- − H, 12). EI-MS *m/z* (%): 316 ($M^+ - 162 - 2H_2O$, 53), 299 ($M^+ + H - 162 - 3H_2O$, 100). ^{1}H-NMR: given in Tables I and II. ^{13}C-NMR: given in Table III. *Anal.* Calcd for $C_{27}H_{46}O_9 \cdot 1/2H_2O$: C, 61.93; H, 9.05. Found: C, 61.80; H, 8.81.

Enzymic Hydrolysis of 1——A suspension of Molsin (protease type XIII)[7] (200 mg) in 0.2 M citric acid–0.2 M Na_2HPO_4 buffer (pH 4.0, 6 ml) was added to a solution of **1** (23.8 mg) in EtOH (1 ml). The reaction mixture was stirred at 37 °C for 40 h, then poured into H_2O, and extracted four times (100 ml × 1 and 30 ml × 3) with AcOEt. The combined AcOEt layer was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The residue (11.4 mg) was recrystallized from MeOH to give the genuine aglycone (**2**) (4.3 mg), colorless plates of mp 281—283 °C (lit. 9, mp 280—282 °C), $[\alpha]_D$ +22.0° ($c=0.25$). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2920, 1440, 1370, 1035. EI- and accurate MS *m/z* (%): 334.250 ($M^+ - H_2O$, Calcd for $C_{21}H_{34}O_3$ 334.251, 22), 316.240 ($M^+ - 2H_2O$, Calcd for $C_{21}H_{32}O_2$ 316.240, 100) 298.229 ($M^+ - 3H_2O$, Calcd for $C_{21}H_{30}O$ 298.230, 72), 290.225 [M^+ − side chain (=C_2H_5O) − H_2O, Calcd for $C_{19}H_{30}O_2$ 290.225, 35], 272.212 (M^+ − side chain − $2H_2O$, Calcd for $C_{19}H_{28}O$ 272.214, 14), 258.198 (M^+ − side chain $-2H_2O-CH_3$, Calcd for $C_{18}H_{26}O$ 258.198, 40). FD-MS *m/z* (%): 353 (M^++H, 3), 334 ($M^+ - H_2O$, 100), 290 ($M^+ + H - H_2O$ − side chain, 78). ^{1}H- and ^{13}C-NMR: given in Tables I and III, respectively.

Diacetonide (3) of 2——The steroidal aglycone **2** (5.5 mg) was stirred with a catalytic amount of 60% $HClO_4$ (2 drops) in acetone (3 ml) at room temperature for 3 h. The reaction mixture was poured into 5% aqueous $NaHCO_3$,

and extracted with C_6H_6. The C_6H_6 layer was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The residue was purified on a silica gel column with *n*-hexane–AcOEt (8 : 1) as the eluant to afford the corresponding diacetonide (**3**) (2.6 mg) in a pure form, colorless needles of mp 174—176 °C (MeOH) (ref. 9, mp 182—184 °C). FD-MS *m/z* (%): 432 (M^+, 11), 418 ($M^+ + H - CH_3$, 100). ^{1}H-NMR: given in Table I.

Methanolysis of 1——A solution of **1** (5 mg) in 5% anhydrous HCl–MeOH (1.3 ml) was refluxed for 4 h. The reaction mixture was neutralized with Ag_2CO_3. The inorganic precipitate was filtered off and the filtrate was concentrated under reduced pressure to give a residue, from which methyl glucoside was identified by HPLC in two different solvent systems [*n*-BuOH–pyridine–H_2O (75 : 15 : 10) and AcOEt–iso-PrOH–H_2O (32 : 12 : 1)]. Furthermore, the residue was trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide–pyridine, and subjected to GLC analysis to demonstrate the presence of methyl glucoside.

References and Notes

1) Part III: A. Inada, M. Kobayashi, and T. Nakanishi, *Chem. Pharm. Bull.*, **36**, 609 (1988).
2) This work was presented at the Japanese-United States Congress of Pharmaceutical Sciences, Honolulu, Dec. 1987, Poster Abstracts, p. 207.
3) "Dictionary of Chinese Crude Drugs (Zhong-Yao-Da-Ci-Dian in Chinese)," ed. by Chiang Su New Medical College (Jiang-Su-Xin-Xue-Yuan), Shanghai Scientific Technologic Publisher (Shang-Hai-Ren-Min-Chu-Ban-She), Shanghai, 1977, p. 2431.
4) *a*) S. Kitamura and G. Murata, "Coloured Illustrations of Woody Plants of Japan," Vol. I, Hoikusha Publishing Co., Ltd., Osaka, 1976, p. 308; *b*) "Hirokawa's Dictionary of Medicinal Plants," ed. by M. Konoshima, S. Shibata, T. Shimomura, and T. Higashi, Hirokawa Publishing Co., Tokyo, 1980, p. 193.
5) T. Nakanishi, H. Terai, M. Nasu, I. Miura, and K. Yoneda, *Phytochemistry*, **21**, 1373 (1982).
6) S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).
7) H. Fujimoto, K. Suzuki, H. Hagiwara, and M. Yamazaki, *Chem. Pharm. Bull.*, **34**, 88 (1986).
8) C. L. Van Antwerp, H. Eggert, G. D. Meakins, J. O. Miners, and C. Djerassi, *J. Org. Chem.*, **42**, 789 (1977).
9) M. Nakatani, H. Takao, I. Miura, and T. Hase, *Phytochemistry*, **24**, 1945 (1985).
10) This signal assignment (δ 1.00) for 1α-H in **3** was also verified by a ^{13}C–^{1}H COSY experiment.
11) The 13-methyl group is affected by the great anisotropy of the 20β-oxygen (in a 1,3-diaxial relationship to the methyl) and resonates at δ 1.08 (Table I), this fact is indicative of a chair form of the six-membered acetonide ring, rather than the other conformers of the ring.
12) W. Klyne, *Biochem. J.*, **47**, 51 (1950); T. Suga, T. Aoki, Y. Kawada, S. Ohta, and E. Ohta, *Phytochemistry*, **23**, 1297 (1984).