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Phytochemical Studies on Meliaceous Plants. III.[1] Structures of Two New Pregnane Steroids, Toosendansterols A and B, from Leaves of *Melia toosendan* SIEB. *et* ZUCC.[2]

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Two new pregnane steroids, named toosendansterols A (**1**) and B (**2**), have been isolated, together with loliolide (**3**), from leaves of *Melia toosendan* SIEB. *et* ZUCC. (Meliaceae). Based on chemical, physicochemical, and spectral evidence, the structures of **1** and **2** have been elucidated as 3β,20*S*-dihydroxy-5α-pregnan-16-one and 3α,20*S*-dihydroxy-5α-pregnan-16-one, respectively. In addition, this is the first identification of **3** in meliaceous plants.

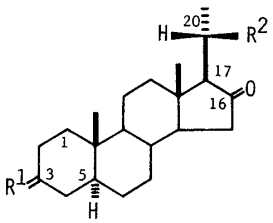
Keywords——*Melia toosendan*; Meliaceae; leaf; pregnane steroid; toosendansterol A; toosendansterol B; loliolide

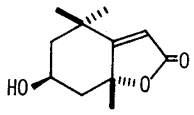
Leaves of *Melia toosendan* SIEB. *et* ZUCC.[3] [*M. azedarach* L. var. *toosendan* (SIEB. *et* ZUCC.) MAKINO[4]] (Meliaceae) are used as a Chinese crude drug, Lian-Ye in Chinese (Ren-Yo in Japanese) and have so far been used in China as a anodyne for malaria, uredo, sting, stomach-ache due to roundworms, *etc.*,[3] and as an insecticide.[3,4b] In our phytochemical studies on meliaceous plants, we have recently identified a new apotirucallane triterpene[1b] and a new derivative of tirucallane triterpene[1a] from fruits of *M. toosendan*.

In our continuing investigation on chemical components in leaves of the plant, two new pregnane steroids, toosendansterols A (**1**) and B (**2**), were isolated, after chromatographic and high-pressure liquid chromatographic (HPLC) separation of the petroleum ether-soluble part of the methanol extracts, and their structures were determined on the basis on spectroscopic, chemical, and physicochemical evidence. A known compound of terpenoid origin, loliolide (**3**), isolated from the chloroform-soluble part, was also identified (see Experimental).[5]

Both of toosendansterols A (**1**) [mp 141—144 °C, $[\alpha]_D$ −108.6° ($CHCl_3$)] and B (**2**) [mp 174—176 °C, $[\alpha]_D$ −54.6° ($CHCl_3$)] showed hydroxyl (**1**, 3500 cm^{-1}; **2**, 3450 cm^{-1}) and carbonyl (**1** and **2**, 1720 cm^{-1}) absorptions in the infrared (IR) spectra. Electron impact mass spectra (EI-MS) and accurate mass spectroscopic data showed **1** and **2** to possess the same molecular formula, $C_{21}H_{34}O_3$ (**1**; 334.249 and **2**; 334.250). In addition, both steroids gave a common and significant fragment ion at *m/z* 275 [$M^+ - C_3H_7O$ (18-CH_3 + side chain)] (the base peak), due to a typical and characteristic cleavage in 16-ketosteroids.[6]

The proton nuclear magnetic resonance (^{1}H-NMR) spectra of **1** and **2** showed signals ascribed to two tertiary methyls (**1**, δ 0.79 and 0.84; **2**, δ 0.79 and 0.81) (18- and 19-H_3), a secondary methyl [**1**, δ 1.20 (d, J=6.4 Hz); **2**, δ 1.21 (d, J=6.3 Hz)] (21-H_3), and two methines bearing an oxygen function [**1**, δ 3.61 (m) and 3.98 (dq, J=8.6, 6.4 Hz); **2**, δ 4.06 (br s) and 3.98 (dq, J=9.0, 6.3 Hz)] (H-3 and H-20). The chemical shifts, multiplicities, and coupling constants of these carbinyl-methines, in conjunction with the presence of the vicinal coupling between 20-H and 17-H (confirmed by the double resonance method) and the mass spectral evidence (*vide supra*), suggested that **1** and **2** could respectively be assigned as 3β,20-dihydroxy- and 3α,20-dihydroxy-5α-pregnan-16-ones.[6b,7] Furthermore, based on the follow-





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1: $R^1 = \beta$-OH, α-H, R^2=OH
1a: $R^1 = \beta$-OAc, α-H, R^2=OAc
2: $R^1 = \alpha$-OH, β-H, R^2=OH

Chart 1

TABLE I. ^{13}C-NMR (100.5 MHz) Data for **1**, **2**, and **1a**, δ (ppm) from Tetramethylsilane in $CDCl_3$

	1	2	1a
C-1	36.72 t	31.93 t	36.47 t
C-2	31.44 t	28.99 t	27.38 t
C-3	71.20 d	66.42 d	73.48 d
C-4	38.09 t	35.83 t	33.91 t
C-5	44.81 d	39.06 d	44.62 d
C-6	28.42 t	28.27 t	28.27 t
C-7	32.15 t	32.10 t	32.02 t
C-8	34.16 d	34.16 d	34.13 d
C-9	54.17 d	54.15 d	54.06 d
C-10	35.66 s	36.25 s	35.66 s
C-11	20.65 t	20.19 t	20.46 t
C-12	38.81 t	38.81 t	38.33 t
C-13	41.90 s	41.92 s	42.06 s
C-14	50.79 d	50.84 d	50.42 d
C-15	38.99 t	38.99 t	39.06 t
C-16	222.91 s	223.02 s	214.92 s
C-17	66.06 d	66.08 d	66.09 d
C-18	13.57 q	13.57 q	13.72 q
C-19	12.33 q	11.19 q	12.20 q
C-20	69.33 d	69.31 d	67.15 d
C-21	21.64 q	21.65 q	20.04 q
$OCOC\underline{H}_3$			21.40 q
			21.40 q
$OC\underline{O}CH_3$			170.50 s
			170.66 s

ing carbon-13 nuclear magnetic resonance (^{13}C-NMR) study, it was indicated that both **1** and **2** contain a 5α-pregnane nucleus. All carbons of **1** and **2** were assigned as shown in Table I with the aid of INEPT experiments and based on the data in ref. 8. The chemical shifts of the carbons on rings A and B of **1** and **2** were in good agreement with those of 3β-hydroxy-5α-cholestanol and 3α-hydroxy-5α-cholestanol, respectively.[8]

The whole structure of **1** was established as follows. On acetylation, **1** furnished the corresponding diacetate (**1a**), mp 148—151 °C, $[\alpha]_D$ −82.8° ($CHCl_3$), which was identical with an authentic sample of synthetic 3β,20*S*-diacetoxy-5α-pregnan-16-one[9] by direct comparison of melting point (mixed mp),[10] IR (KBr), ^{1}H- and ^{13}C-NMR spectra, and thin layer chromatography (TLC). Thus, toosendansterol A (**1**) was identified as 3β,20*S*-dihydroxy-5α-pregnan-16-one.

Finally, a detailed comparison of the spectral data [IR, mass, ^{1}H- and ^{13}C-NMR (Table I)] of **2** with those of **1** indicated that **2**, as well as **1**, was of the 20*S* configuration, and the structure of toosendansterol B (**2**) was established as 3α,20*S*-dihydroxy-5α-pregnan-16-one, *i.e.*, the epimer of **1** at C-3.

Steroids **1** and **2** are both new compounds, and this is the first identification of loliolide (**3**) in meliaceous plants.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were run with a JASCO A-302 instrument. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.5 MHz) spectra were measured with a JEOL JNM-GX400 spectrometer with $CDCl_3$ as a solvent and tetramethylsilane as an internal standard. EI-MS and accurate MS were obtained with a JEOL JNM-DX300 spectrometer at 30 eV, and field

desorption (FD)-MS with the same spectrometer using carbon emitters under the following conditions: accelerating voltage, 3 kV; emitter current, 14 mA; chamber at room temperature. Optical rotations were determined for solutions in $CHCl_3$ on a JASCO DIP-140 digital polarimeter. For column chromatography and TLC, Kieselgel 60 (Merck; 230—400 mesh) and precoated silica gel plates (Merck HF-254) were used, respectively. Low-pressure liquid chromatography was performed on a Yamazen instrument with a Fluid model RP-SY pump and a prepacked Lobar LiChroprep Si-60 (40—63 μm; 25 mm × 31 cm) column was used with $CHCl_3$–MeOH (19 : 1) as an eluting solvent. Preparative HPLC was carried out on a Waters instrument with an M 6000A pump, a U6K septumless injector, and a series R 401 differential refractometer. A micro-bonded silica-packed column (Waters μ-Porasil; 7.8 mm × 30 cm) was used, with an eluant ($CHCl_3$) flow of 3 ml·min^{-1}.

Plant Material——Leaves of *M. toosendan* were collected in 1985 at the Medicinal Plant Garden of Osaka University (Faculty of Pharmaceutical Sciences, Suita, Osaka, Japan) and identified by Dr. K. Yoneda, Faculty of Pharmaceutical Sciences, Osaka University.

Isolation of Steroids 1 and 2——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 extracts (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 (57.5 g) obtained from the petroleum ether layer was twice subjected to column chromatography [1st column, silica gel 500 g (eluant $CHCl_3$); 2nd column, silica gel 100 g (eluant, benzene–acetone 4 : 1)] and a fraction (0.5 g) containing the steroids was separated. This fraction was further separated by low-pressure liquid chromatography and then by preparative HPLC, and steroids **1** and **2** were eluted in that order. Both steroids obtained were further purified by repeated HPLC separation to give pure **1** (15 mg) and **2** (8 mg).

Toosendansterol A (1)——Colorless fine plates of mp 141—144 °C (hexane–Et_2O), $[\alpha]_D$ −108.6° (c=0.15). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (OH), 2925, 1720 (CO), 1405, 1275, 1070, 1035. EI- and accurate MS *m/z* (%): 334.249 (M^+, calcd for $C_{21}H_{34}O_3$ 334.251, 22), 319.232 (M^+-CH_3, calcd for $C_{20}H_{31}O_3$ 319.227, 12), 316.244 (M^+-H_2O, calcd for $C_{21}H_{32}O_2$ 316.240, 17), 301.216 ($M^+-CH_3-H_2O$, calcd for $C_{20}H_{29}O_2$ 301.217, 20), 275.205 [$M^+-C_3H_7O$ (side chain + 18-CH_3), calcd for $C_{18}H_{27}O_2$ 275.201, 100]. ^{1}H-NMR δ: 0.79, 0.84 (3H each, both s, 18-H_3, 19-H_3), 1.20 (3H, d, J=6.4 Hz, 21-H_3), 1.82 (1H, d, J=8.6 Hz, 17α-H), 3.61 (1H, m, 3α-H), 3.98 (1H, dq, J=8.6, 6.4 Hz, 20-H), 4.43 (1H, br s, 20-OH). ^{13}C-NMR: given in Table I.

Toosendansterol B (2)——Colorless plates of mp 174—176 °C (hexane–Et_2O), $[\alpha]_D$ −54.6° (c=0.15). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH), 2930, 1720 (CO), 1405, 1210. EI- and accurate MS *m/z* (%): 334.250 (M^+, calcd for $C_{21}H_{34}O_3$ 334.251, 25), 319.226 (M^+-CH_3, calcd for $C_{20}H_{31}O_3$ 319.227, 23), 316.236 (M^+-H_2O, calcd for $C_{21}H_{32}O_2$ 316.240, 23), 301.217 ($M^+-CH_3-H_2O$, calcd for $C_{20}H_{29}O_2$ 301.217, 25), 275.202 [$M^+-C_3H_7O$ (side chain + 18-CH_3), calcd for $C_{18}H_{27}O_2$ 275.201, 100]. ^{1}H-NMR δ: 0.79, 0.81 (3H each, both s, 18-H_3, 19-H_3), 1.21 (3H, d, J=6.3 Hz, 21-H_3), 1.83 (1H, d, J=9.0 Hz, 17α-H), 3.98 (1H, dq, J=9.0, 6.3 Hz, 20-H), 4.06 (1H, br s, 3β-H), 4.44 (1H, br s, 20-OH). ^{13}C-NMR: given in Table I.

Acetylation of 1——A solution of **1** (9 mg) in pyridine (3 ml) and Ac_2O (1.5 ml) was left standing overnight at 37 °C, poured into cold water, and extracted with Et_2O. After usual work-up, the residual product was purified on silica gel column with $CHCl_3$ as the eluant to give **1a** (5.5 mg), colorless fine crystals of mp 148—151 °C (MeOH) (authentic sample: mp 154—157 °C[9,10]; mixed mp 150—154 °C), $[\alpha]_D$ −82.8° (c=0.20). IR ν_{max}^{KBr} cm^{-1}: 2920, 1720, 1375, 1245, 1025 (in Nujol[9]). EI-MS *m/z* (%): 358 (M^+-AcOH, 32), 343 (M^+-CH_3-AcOH, 67), 298 (M^+-2AcOH, 100), 283 (M^+-CH_3-2AcOH, 62). FD-MS *m/z* (%): 419 (M^++H, 100), 418 (M^+, 31). ^{1}H-NMR δ: 0.78, 0.85 (3H each, both s, 18-H_3, 19-H_3), 1.36 (3H, d, J=6.4 Hz, 21-H_3), 2.02, 2.03 (3H each, both s, OCOC$\underline{H}_3$ × 2), 4.70 [1H, m(octet-like), 3α-H], 5.12 (1H, dq, J=8.6, 6.4 Hz, 20-H). ^{13}C-NMR: given in Table I.

Identification of Loliolide (3)——The $CHCl_3$-soluble part (43.5 g) of the MeOH extracts was chromatographed on silica gel (435 g), eluting successively with $CHCl_3$ and $CHCl_3$–MeOH (100 : 1). A fraction (1.7 g) containing **3**, obtained by elution with $CHCl_3$, was further separated by low-pressure liquid chromatography and then by preparative HPLC to give **3** (28 mg), mp 147.5—150.5 °C (Et_2O) (ref. 5*b*, mp 148—149 °C), $[\alpha]_D$ −97.9° (c=0.25) (ref. 5*b*, $[\alpha]_D$ −92° (c=1.1, $CHCl_3$)). The melting points, optical rotation ($CHCl_3$), IR (Nujol), EI-MS, and ^{1}H-NMR spectra of **3** were consistent with the published data for loliolide.[5]

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