PHYTOCHEMICAL STUDIES ON MELIACEOUS PLANTS. V. [1] STRUCTURE OF A NEW APOTIRUCALLANE-TYPE TRITERPENE, 21-O-METHYL-TOOSENDANPENTOL FROM FRUITS OF *MELIA TOOSENDAN* SIEB. ET ZUCC.[#]

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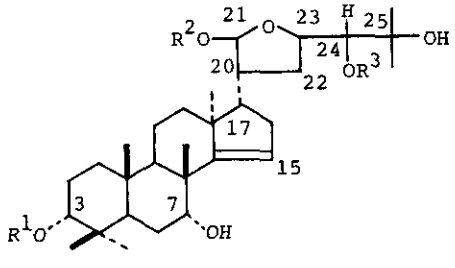
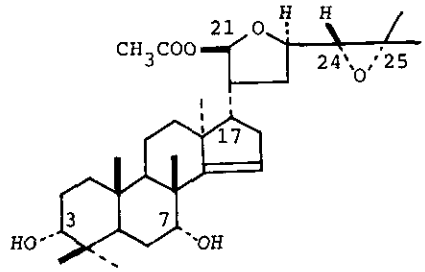
Abstract — 21-O-Methyltoosendanpentol (1), a new apotirucallane-type triterpene, has been isolated from fruits of *Melia toosendan* Sieb. et Zucc. (Meliaceae). The structure of 1 has been established by lines of chemical and spectral evidence.

The air-dried fruit of *Melia toosendan* Sieb. et Zucc.[2] [*M. azedarach* L. var. *toosendan* (Sieb. et Zucc.) Makino[3]] (Meliaceae), i.e. a Chinese crude drug named Chuan-Lian-Zi (Sen-Ren-Si in Japanese) has so far been used in China as an anodyne for stomach-ache due to roundworms, gripe, etc,[2,3a] and also as a vermicide.[2,3b] Recently, we have investigated terpenic components from fruits of the plant and identified the new apotirucallane-type triterpene 21-O-acetyltoosendantriol (2) from methanol extracts of the fruits.[4] Further detailed examination on the methanol extracts has led us to the isolation of another new apotirucallane-type triterpene named 21-O-methyltoosendanpentol (1).

Repeated chromatographic and hplc separation of the chloroform layer from the methanol extracts (see EXPERIMENTAL) gave 1, colorless fine needles, mp 106-108°C, $[\alpha]_D$ -52.1° (MeOH), which has the molecular formula $C_{31}H_{52}O_6$, based on the elemental analysis and the $[M - H]^-$ ion peak (100 %) at m/z 519 in the negative ion fab-ms. The hrms of 1 gives a significant and intense fragment $C_{22}H_{34}O_2$ at m/z 330.255 (100 %), due to the loss of the side chain part and a hydrogen. We have already encountered this characteristic cleavage (M^+ - side chain - H) in the apotirucallane (2)[4] and observed the fragment of the same composition at m/z 330.256. The ^{1}H-nmr spectrum of 1 showed signals due to seven tertiary methyls, a methoxy methyl [δ 3.36 (s)], four protons bearing an oxygen function [δ 3.25 (dd, J= 8.5, 1.8 Hz), 3.41 (m), 3.91 (m), 4.25 (ddd, J= 10.7, 4.9, 1.8 Hz)], an olefinic proton

[#] Dedicated to Sir Professor D. H. R. Barton on the occasion of his 70th birthday.





1 : $R^1 = R^3 = H$, $R^2 = CH_3$
21-O-methyltoosendanpentol

1a : $R^1 = R^3 = COCH_3$, $R^2 = CH_3$

3 : $R^1 = COCH_3$, $R^2 = H$ (α-oriented OH)
$R^3 = H$

2 : 21-O-acetyltoosendantriol[4]

Table I. ^{1}H-Nmr (400 MHz) Data[a] for 1, 1a, and 2, δ (ppm) from TMS in $CDCl_3$

	1	1a	2[4]
3β-H	3.41(m)	4.66(t,2.7)	3.40(m)
7β-H	3.91(m)	3.91(t,2.6)	3.92(m)
15-H	5.44(m)	5.44(m)	5.47(m)
20α-H	2.34(m)	2.35(m)	2.37(m)
21-H	4.80(d,3.7)	4.84(d,3.7)	6.24(d,4.0)
23-H	4.25(ddd,10.7, 4.9,1.8)	4.44(ddd,10.7, 4.6,2.1)	3.92(m)[c]
24-H	3.25(dd,8.5, 1.8)[b]	4.87(d,2.1)	2.67(d,7.5)
24-OH	2.56(d,8.5)	----	----
25-OH	2.80(br s)	3.20(br s)	----
OCH_3	3.36(s)	3.37(s)	----
$OCOCH_3$	----	2.07,2.18 (all s)	2.06(s)
CH_3	0.86,0.90, 0.95,1.05, 1.06,1.26, 1.30(all s)	0.86,0.90, 0.91,1.06, 1.09,1.20, 1.36(all s)	0.84,0.89, 0.94,1.03, 1.05,1.29, 1.33(all s)

a) Multiplicities and coupling constants (Hz) are given in parentheses. b) Becomes d (J= 1.8) after D_2O exchange. c) Multiplicity and coupling constant were obscure, due to partial overlap.

Table II. ^{13}C-Nmr (100.5 MHz) Data[a] for 1 and 2, δ (ppm) from TMS in $CDCl_3$

	1	2
C-1	32.83(t)[b]	32.60(t)[b]
C-2	25.05(t)	25.11(t)
C-3	76.20(d)	76.17(d)
C-4	37.07(s)	37.08(s)
C-5	40.51(d)	40.55(d)
C-6	23.74(t)	23.85(t)
C-7	72.30(d)	72.35(d)
C-8	44.44(s)	44.49(s)
C-9	41.71(d)	41.60(d)
C-10	37.72(s)	37.79(s)
C-11	16.27(t)	16.30(t)
C-12	32.62(t)[b]	32.62(t)[b]
C-13	46.98(s)	46.73(s)
C-14	162.43(s)	162.37(s)
C-15	119.20(d)	119.25(d)
C-16	34.71(t)	35.09(t)
C-17	57.54(d)	52.64(d)
C-20	45.90(d)	44.32(d)
C-21	109.55(d)	96.77(d)
C-22	33.80(t)	31.48(t)
C-23	76.90(d)	79.75(d)
C-24	75.59(d)	66.79(d)
C-25	73.07(s)	57.11(s)
CH_3	15.24(q)	15.23(q)
	19.32(q)	19.38(q)
	22.15(q)	19.59(q)
	26.37(q)	22.14(q)
	26.49(q)	24.94(q)
	27.73(q)	27.91(q)
	28.07(q)	28.07(q)
OCH_3	55.60(q)	----
$OCO\underline{C}H_3$	----	21.48(q)
$O\underline{C}OCH_3$	----	169.88(s)

a) Multiplicities are given in parentheses. b) May be interchanged in each column.

[δ 5.44 (m)], and a hemiacetal proton [δ 4.80 (d, J= 3.7 Hz)] (Table I). These lines of spectral evidence suggest that 1 as well as 2 is the member of the apotirucallane-type triterpene. Acetylation of 1 with Ac_2O and pyridine furnished a diacetate (1a), a white powder, $[\alpha]_D$ -32.0° ($CHCl_3$), which still showed hydroxy absorptions at 3660 and 3450 cm^{-1} in the ir spectrum, suggesting that 7α- and 25-hydroxyls in 1 are resistant to acetylation under normal conditions.[5,6] Based on a detailed comparison of spectral data [ir, ms, and ^{1}H-nmr (Table I)] of 1 and 1a with those of 2, it was inferred that 1 as well as 2 is an apotirucallane with 3α- and 7α-hydroxyls, whereas 1 carries an acetal methoxy group at C_{21} and a vicinal (C_{24}-C_{25}) glycol on its side chain, instead of a hemiacetal acetoxy at C_{21} and an epoxide (C_{24}-C_{25}) ring on the side chain of 2. This inferred structure (1) was further confirmed by the following ^{13}C-nmr study (Table II), etc. All carbons from C-1 to C-16 of 1 closely coincided with those of 2 in both chemical shifts and multiplicities, corroborating that 1 and 2 have the same nucleus with 3α- and 7α-hydroxyls though they are different in their side chain structures. The side chain structure of 1 was deduced as follows. Atoms C-24 and C-25 of 1 resonated at δ 75.59 and 73.07 ppm, respectively, chemical shifts of which are in agreement with those (C-24, δ 75.2 and C-25, δ 73.7 ppm, respectively) of the corresponding carbons reported for compound C (3)[6] with a side chain including a hemiacetal ring and a vicinal (C_{24}-C_{25}) glycol system. Furthermore, in the ^{1}H-nmr (Table I) spectrum of 1, the 24-H signal consistent with that (δ 3.27) of 3[6] in chemical shifts was also observed along with the 25-OH singlet. These ^{13}C- and ^{1}H-nmr evidence confirmed the presence of the vicinal (C_{24}-C_{25}) glycol on the side chain of 1. Finally, a methoxy group in 1 is reasonably assigned to an acetal methoxy at C-21 on the side chain, based on the so far accumulated chemical and spectral evidence. Thus, the structure of 21-O-methyltoosendanpentol is now defined as 1.

EXPERIMENTAL

Melting points are uncorrected. The following instruments were used for obtaining the physical data; optical rotation, JASCO DIP-140 digital polarometer; ir spectra, JASCO A-302 instrument; mass, hrms, and negative ion fab-ms (accelerating voltage, 2-3 kV; matrix, triethanolamine; collision gas, Xe), JEOL JNM-DX300; ^{1}H- (400 MHz) and ^{13}C-nmr (100.5 MHz), JEOL JNM-GX400 with $CDCl_3$ as a solvent and TMS as an internal standard. Preparative hplc was performed on a Kusano instrument with a KPW-10 micro-pump and a Shodex SE-31 differential refractometer. A Kusano Si-10

silica column [10 cm x 22 mm i.d.; mobile phase, $CHCl_3$-MeOH (15:1), flow rate; 3 ml/min] was used.

Plant Material

Fruits of M. toosendan were collected in 1983 at the Medicinal Plants Garden of Osaka University (Faculty of Pharmaceutical Sciences, Suita, Osaka, Japan).

Isolation of 1 and 2

Air-dried and crushed fruits (2.0 kg) were extracted twice with MeOH (6 l) at r.t. for 10 days. After evaporation of the solvent, the MeOH extracts (262.5 g) were suspended in H_2O, and extracted successively with petroleum ether (500 ml x 3) and $CHCl_3$ (500 ml x 3). The $CHCl_3$ layer was concentrated to give a residue (21.7 g), a portion of which (20.0 g) was repeatedly chromatographed on silica gel. A fraction containing 2 (320 mg) by eluting with hexane-AcOEt (1:1) and a fraction containing 1 (60 mg) with hexane-AcOEt (1:2) were separated in that order. Each fraction was further purified by preparative hplc separation to give pure 1 (30 mg) and 2 (120 mg).

21-O-Methyltoosendanpentol (1)

Colorless fine needles of mp 106-108°C (hexane-acetone), $[\alpha]_D$ -52.1° (c, 0.36 in MeOH). IR ν_{max}^{KBr} cm^{-1}: 3450, 2925, 1460, 1380, 1035. Ms and hrms m/z (%): 431 (1), 399 (6), 330.255 (M^+ - side chain - H, calcd for $C_{22}H_{34}O_2$ 330.257, 100),[4] 312 (15), 235 (5), 160 (19). Negative ion fab-ms m/z (%): 519 [$(M - H)^-$, 100]. ^1H- and ^{13}C-nmr data are given in Tables I and II, respectively. Anal Calcd for $C_{31}H_{52}O_6$: C, 71.50; H, 10.07. Found: C, 71.52; H, 10.47.

Acetylation of 1

A solution of 1 (10 mg) in pyridine (3 ml) and Ac_2O (0.5 ml) was left standing overnight at r.t., poured into cold water, and extracted with AcOEt. The AcOEt was dried over $MgSO_4$ and after evaporation of the solvent, the residual product was chromatographed on silica gel to give 1a (6 mg), a white powder, $[\alpha]_D$ -32.0° (c, 0.22 in $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3660 (OH), 3450 (OH), 2930, 1720, 1370, 1230, 1020. Negative ion fab-ms m/z (%): 603 $\{[M(C_{35}H_{56}O_8) - H]^-$, 5$\}$. ^1H-nmr data is given in Table I.

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