THE STRUCTURES OF TWO NEW IONONE GLUCOSIDES FROM MELIA TOOSENDAN AND A NOVEL TYPE OF SELECTIVE BIO-OXIDATION

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The structures of two new ionone glucosides, melia-ionosides A and B, isolated from leaves of Melia toosendan were determined based on the combined evidence of chemical, spectral, and X-ray studies. In addition, a novel type of selective bio-oxidation was found during the chemical study.

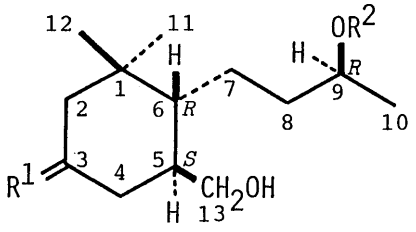
KEYWORDS Melia toosendan; Meliaceae; leaf; ionone glucoside; melia-ionoside A; melia-ionoside B; Molsin; bio-oxidation

A Chinese crude drug named Lian-Ye (Ren-yoh in Japanese) is the air-dried leaves of Melia toosendan Sieb. et Zucc.[1] (Meliaceae) and is used in China as an anodyne for malaria, uredo, sting, stomach-ache caused by roundworms, etc., and as an insecticide.[1] During the course of our phytochemical research on meliaceous plants, we have isolated two new ionone glucosides named as melia-ionosides A (**1**) and B (**2**), after chromatographic and HPLC separation of the MeOH extract from the leaves of M. toosendan (0.007 and 0.003% yields from the extr., respectively).

Both **1**, a white powder, $[\alpha]_D^{20}$ -35.1°(c 0.43, Py.) and **2**, a white powder, $[\alpha]_D^{20}$ -9.0°(c 0.15, Py.) gave hydroxy-bands (KBr; 3350 and 1075 cm^{-1}) in the IR spectra and had the same molecular formula, $C_{19}H_{36}O_8$, confirmed by the elemental analyses and the negative ion FAB-MS data [$(M-H)^-$(100%); m/z 391]. It was inferred from detailed ^1H- and ^{13}C-NMR assignments (Tables I and II), performed with the aid of ^1H-^1H COSY, NOESY, INEPT, ^1H-^{13}C COSY techniques, that both **1** and **2** are comprised of a β-D-glucosyl residue[2] and an ionone (**3**) as the common aglycone, i.e., both are isomers of ionone glucosides which differ from one another in their glucosyl positions on the aglycone.

When refluxed (4h) with 10% H_2SO_4-MeOH (1:2), both **1** and **2** afforded the common and genuine aglycone, the ionone (**3**), $C_{13}H_{26}O_3$ (M^+=230.188), colorless oil, $[\alpha]_D^{20}$ -6.8° (c 0.10, Py.) and one mole of D-glucose (identified by PPC, TLC, and GLC). The detailed ^1H- and ^{13}C-NMR assignments (Tables I and II) indicated that the relative structure is shown in (**3**) or its antipode, except for the configuration of the 9-OH group on the side chain.

The whole relative structure of **3**, including the 9-OH configuration, was determined by a combination of the following chemical study and X-ray analysis. On the way to the chemical derivation, a novel type of selective bio-oxidation was found, this is also reported here. When incubated with Molsin (protease type XIII from Aspergillus saitoi),[3] the ionone (=triol)(**3**) gave the corresponding 3-keto-derivative (**4**), $C_{13}H_{24}O_3$ (M^+=228.172), colorless oil, $[\alpha]_D^{20}$ +24.6° (c 0.35,$CHCl_3$) in 84% yield as a result of the novel selective bio-oxidation reaction, in which only the axial 3-OH group among three primary and secondary hydroxyls in **3** was oxidized. In a similar treatment with Molsin, both glucosides **1** and **2** also afforded **4** (61 and 78% yield, respectively), in which the usual hydrolysis followed by the selective oxidation might take place.



1: R^1 = α-O-β-D-glucopyranosyl, β-H (S), R^2 = H
2: R^1 = α-OH, β-H (S), R^2 = β-D-glucopyranosyl
3: R^1 = α-OH, β-H (S), R^2 = H
4: R^1 = O, R^2 = H
5: R^1 = $NNHC_6H_3(NO_2)_2$, R^2 = H

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

	1[b]	**2**[b]	**3**[c]
2α-H	1.95,dd,14.0,2.8	1.88,dd,13.7,3.4	1.92,dd,13.8,3.1
2β-H	1.42,dd,14.0,3.4	1.47,dd,13.7,3.4	1.52,dd,13.8,3.4
3β-H	4.42,tt,3.4,2.8	4.43,quin,3.4	4.47,tt,3.4,3.1
4α-H	2.67,ddd,11.9,2.8,1.8	2.47,ddd,13.7,3.4,2.4	2.52,ddd,13.4,3.1,2.4
4β-H	1.6[d]	1.8[d]	1.9[d]
5α-H	2.27,m,Wh/2=19.0[e]	2.30,m,Wh/2=19.0[e]	2.38,m,Wh/2=19.0[e]
6β-H	1.10,ddd,10.4,5.2,2.6	1.15,ddd,10.4,5.5,1.8	1.25,ddd,10.6,5.2,2.6
7-H_2	1.5[d] and 1.7[d]	1.6[d] and 1.8[d]	1.7[d] and 1.8[d]
8-H_2	1.6[d] and 1.98,m	1.7[d] and 2.12,m	1.7[d] and 2.06,m
9α-H	4.0[d]	4.1[d]	4.06,m
13-H_2	3.86,dd,10.7,6.7	3.91,dd,10.7,6.1	3.99,dd,10.3,6.3
	4.14,dd,10.7,3.1	4.09,dd,10.7,3.4	4.18,dd,10.3,3.2
10-H_3	1.38,d,6.4	1.40,d,6.1	1.40,d,6.1
11-H_3	1.22,s	1.32,s	1.39,s
12-H_3	0.94,s	1.02,s	1.05,s
1'-H	4.95,d,7.9	4.98,d,7.9	
2'-H	4.01,dd,7.9,8.9	4.02,dd,7.9,8.9	
3'-H	4.28,t,8.9	4.26,t,8.9	
4'-H	4.19,dd,8.9,9.5	4.19,dd,8.9,9.2	
5'-H	3.92,ddd,9.5,5.5,2.1	3.97,ddd,9.2,5.5,2.4	
6'-H_2	4.33,dd,11.9,5.5	4.35,dd,11.9,5.5	
	4.52,dd,11.9,2.1	4.54,dd,11.9,2.4	

a) Chemical shifts are in δ values relative to internal TMS, and are followed by multiplicities and coupling constants (Hz). Assignments for these compounds were made with the aid of ^{1}H-^{1}H COSY, NOESY, and ^{13}C-^{1}H COSY methods. b) In Py.-d_5 with few drops of D_2O. c) In Py.-d_5. d) Both multiplicity and coupling constant were obscure, due to partial overlap. e) Peak width at half height (Wh/2) is in Hz.

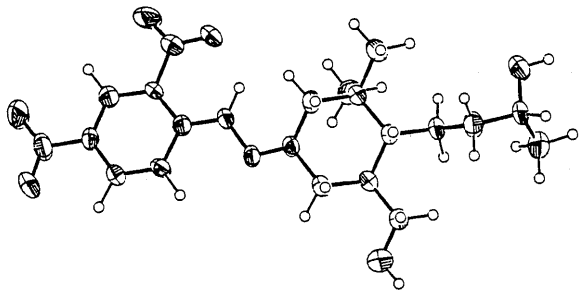


Fig. 1

Table II. ^{13}C-NMR (100.5 MHz) Data for **1**-**4** (δ_C, ppm from TMS in Py.-d_5)[a]

	1[b]	**2**[b]	**3**	**4**
1-C	34.70 (s)	34.73 (s)	34.79 (s)	39.10 (s)
2-C	46.36 (t)	47.81 (t)	48.09 (t)	56.50 (t)
3-C	73.92 (d)	66.55 (d)	66.70 (d)	211.19 (s)
4-C	34.23 (t)	38.39 (t)	38.72 (t)	45.39 (t)
5-C	37.78 (d)	37.78 (d)	38.13 (d)	44.48 (d)
6-C	48.22 (d)	48.68 (d)	48.62 (d)	46.49 (d)
7-C	25.65 (t)	24.79 (t)	25.93 (t)	25.74 (t)
8-C	41.73 (t)	38.30 (t)	41.94 (t)	41.45 (t)
9-C	67.55 (d)	76.26 (d)	67.59 (d)	67.47 (d)
10-C	24.44 (q)	21.89 (q)	24.47 (q)	24.44 (q)
11-C	23.59 (q)	23.98 (q)	23.95 (q)	20.90 (q)
12-C	31.71 (q)	31.83 (q)	31.89 (q)	29.93 (q)
13-C	65.15 (t)	65.36 (t)	65.51 (t)	63.83 (t)

a) Assignments and multiplicities (in parentheses) were determined based on INEPT and ^{1}H-^{13}C COSY experiments. b) Data of the D-glucosyl parts in **1** and **2** are as follows. **1**: 102.57 (d, 1'), 75.46 (d, 2'), 78.87 (d, 3'), 71.89 (d, 4'), 78.31 (d, 5'), 62.98 (t, 6') and **2**: 103.52 (d, 1'), 75.20 (d, 2'), 78.35 (d, 3'), 71.68 (d, 4'), 78.14 (d, 5'), 62.75 (t, 6').

As the next step, the ketone (**4**) was, in the usual manner, transformed into the corresponding 2,4-dinitrophenylhydrazone (**5**), $C_{19}H_{28}N_4O_6$ (M^+=408.200), mp 148–150°C (from AcOEt), crystals of which were subjected to a single crystal X-ray analysis; Monoclinic with space group $P2_1$, a = 15.613(3), b = 8.149(1), c = 8.141(1) Å, β = 91.32(2)°, Z = 2, V = 1035.5(3) Å^3, D_X = 1.309, D_M = 1.29(1) Mgm^{-3}. Intensities of 1504 ($F_o \geq 3\sigma F_o$) independent reflections with 2θ values up to 125° were collected on a diffractometer with graphite monochromated Cu-Kα radiation, using the ω–2θ scanning technique. The structure was solved by the direct method using the SAYTAN87 program and refined by the block diagonal least squares method to an R value of 0.062 for all non-hydrogens with anisotropic temperature factors and with isotropic ones for hydrogen atoms, which were found in the difference Fourier map.[4] Figure 1 shows a computer-generated perspective drawing (ORTEP) of the molecule (**5**), including an ionone framework with (5S, 6R, 9R) or (5R, 6S, 9S) configurations. This X-ray analysis also showed that the relative structure for the ketone (**4**) is defined as either the structure (**4**) or its optical antipode. However, the structure (**4**) had already been assigned to nigakialcohol which gives a positive CD Cotton effect ([θ] +310) at 294 nm (c 0.083, EtOH).[5] The CD behavior of the ketone (**4**), [θ] +276 at 294 nm (c 0.086, EtOH) was in agreement with that of nigakialcohol, indicating that the ketone had the absolute structure (**4**).

Furthermore, the accumulated evidence demonstrated that the aglycone (**3**) is assigned to the ionone structure (**3**) with (3S, 5S, 6R, 9R) chiral centers.

Finally, each location of a β-D-glucosyl residue on the aglycone (**3**) was confirmed in both glucosides (**1** and **2**) as follows. In the ^{13}C-NMR spectra (Table II), the C-3 carbon of **1** resonated downfield (by +7.22 ppm) from the corresponding signal for **3**. In contrast the C-2 and C-4 carbons of **1** resonated upfield (by −1.73 and −4.49 ppm, respectively) from those for **3**. But C-9 and both the C-8 and C-10 of **2** were respectively shifted downfield (C-9; +8.67 ppm) and upfield (C-8 and C-10; −3.64 and −2.58, respectively), compared with those for **3**. Besides, in the NOESY spectra of **1** and **2**, there was a typical cross peak between 3β-H and the glucosyl anomeric proton in **1** and between 9α-H and the glucosyl anomeric proton. These NMR studies proved that a β-D-glucosyl moiety is linked at 3α-OH of the aglycone (**3**) in **1** and at 9β-OH of **3** in **2**, respectively. Based on the accumulated evidence, the absolute structures for melia-ionosides A and B are defined as **1** and **2**.

Both glycosides **1** and **2** were found in nature for the first time. Also, their common aglycone (**3**) was a new saturated ionone. To our knowledge, this is the first report about absolute structures for glycosides of new saturated ionones.

REFERENCES AND NOTES

1) "Dictionary of Chinese Crude Drugs," ed. by Chiang Su New Medical College, Shanghai Scientific Technologic Publisher, Shanghai, 1977, p. 2431.
2) With respect to the configuration of glucose in **1** and **2**, the D form must be preferable from the viewpoint of natural occurrence. The β-orientation of the glucosyl linkages in **1** and **2** were confirmed based on large J-values of the respective anomeric protons (Table I) together with chemical shifts for the respective anomeric carbons (Table II).
3) The Molsin used in this work is commercially availed (Sigma Chem. Co., Lot. No. 104F-0124). A typical reaction procedure of the bio-oxidation is as follows. A suspension of Molsin (120 mg) in 0.2 M citric acid–0.2 M Na_2HPO_4 buffer (pH 4.0; 2.5 ml) was added to a solution of **3** (8.2 mg) in EtOH (0.3 ml) and the resultant mixture was stirred at 37°C for 96 h. The ketone (**4**) (6.8 mg; 84% yield) was obtained as a sole oxidation product, together with recovered starting material.
4) Atomic coordinates, structure and temperature factors, bond distances and angles will be deposited with the Cambridge Crystallographic Data Centre.
5) Y. Sugimoto, T. Sakita, T. Ikeda, Y. Moriyama, T. Murae, T. Tsuyuki, and T. Takahashi, Bull. Chem. Soc. Jpn., 52, 3027 (1979).

(Received January 22, 1990)