Phytochemical Studies on Meliaceae Plants. VII.¹⁾ The Structures of Two New Ionone Glucosides from *Melia toosendan* SIEB. et ZUCC. and a Novel Type of Selective Biooxidation by a Kind of Protease

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Two new ionone glucosides, melia-ionosides A and B, were isolated from leaves of *Melia toosendan* SIEB. et ZUCC. (Meliaceae) and their structures were elucidated based on combinations of chemical, spectral, and X-ray analytical studies. In addition, a novel type of selective bio-oxidation by a kind of protease (Molsin) was found during the course of the structural elucidation.

Keywords *Melia toosendan*; Meliaceae; leaf; ionone glucoside; melia-ionoside A; melia-ionoside B; bio-oxidation; Molsin; saturated ionone alcohol

In our continuing phytochemical studies on meliaceae plants, we have already characterized from the leaves of *Melia toosendan* SIEB. et ZUCC.²⁾ (Meliaceae) two pregnane steroids³⁾ and a pregnane glucoside, toosendanoside.⁴⁾ We have also briefly reported the structural elucidation of two new ionone glucosides, melia-ionosides A (**1**) and B (**2**), by means of two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy, X-ray analysis, and chemical reactions in the preceding communication.¹⁾ This paper presents a full account of the isolation and structural elucidation of the glucosides **1** and **2**. A novel type of bio-oxidation found during the course of the structural study is also reported in detail.

Results and Discussion

The *n*-butanol fraction of the methanol extract from the plant material was subjected to repeated and precise separation by column and high-performance liquid chromatography (HPLC) to isolate **1**, a white powder, $[\alpha]_D^{20} -35.1^\circ$ and **2**, a white powder, $[\alpha]_D^{20} -9.0^\circ$ in yields (from the methanol extract) of 0.007 and 0.003%, respectively.

Both **1** and **2** showed hydroxy absorption bands (3350 and 1075 cm^{-1}) in the infrared (IR) spectra and possessed the same molecular formula $\text{C}_{19}\text{H}_{36}\text{O}_8$, based on elemental analyses and the presence of the common $(\text{M}-\text{H})^-$ ion (the base peak) at m/z 391 in the negative ion fast atom bombardment mass (FAB-MS) spectra. As the next step of the structural elucidation, detailed studies of the proton and carbon-13 nuclear magnetic resonance (^1H - and ^{13}C -NMR) spectra of **1** and **2** were performed with the aid of the INEPT (insensitive nuclei enhanced by polarization transfer) method and 2D techniques such as ^1H - ^1H and ^{13}C - ^1H shift-correlated spectroscopy (COSY) and nuclear Over-

hauser effect spectroscopy (NOESY), and as shown in Tables I and II, all protons and carbons of both **1** and **2** were assigned.

Based on the established assignments, it was inferred that both **1** and **2** contain a β -D-glucopyranosyl ($^4\text{C}_1$ form) residue⁵⁾ and an ionone (**3**) as the common aglycone, that is, these ionone glucosides (**1** and **2**) are isomers as regards the glucosyl position on **3**.

On acidic hydrolysis, both glucosides afforded the common and genuine aglycone, the ionone (**3**), $\text{C}_{13}\text{H}_{26}\text{O}_3$, a colorless oil, $[\alpha]_D^{20} -6.8^\circ$ and D-glucose. The ^1H - and ^{13}C -NMR assignments established for **3** (Tables I and II) indicated that the relative structure of the ionone is as shown

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

	1 ^{b)}	2 ^{b)}	3 ^{c)}
2 α -H	1.95, dd, 14.0, 2.8	1.88, dd, 13.7, 3.4	1.92, dd, 13.8, 3.1
2 β -H	1.42, dd, 14.0, 3.4	1.47, dd, 13.7, 3.4	1.52, dd, 13.8, 3.4
3 β -H	4.42, tt, 3.4, 2.8	4.43, quin, 3.4	4.47, tt, 3.4, 3.1
4 α -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
4 β -H	1.6 ^{d)}	1.8 ^{d)}	1.9 ^{d)}
5 α -H	2.27, m, $W_{h/2} = 19.0$ ^{e)}	2.30, m, $W_{h/2} = 19.0$ ^{e)}	2.38, m, $W_{h/2} = 19.0$ ^{e)}
6 β -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)} 1.7 ^{d)}	1.6, ^{d)} 1.8 ^{d)}	1.7, ^{d)} 1.8 ^{d)}
8-H ₂	1.6, ^{d)} 1.98, m	1.7, ^{d)} 2.12, m	1.7, ^{d)} 2.06, m
9 α -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 ^1H - ^1H COSY, NOESY, and ^{13}C - ^1H COSY methods. b) In Py- d_5 with a few drops of D_2O . c) In Py- d_5 . d) Both multiplicity and coupling constant were obscure, due to partial overlapping. e) Peak width at half height ($W_{h/2}$) is given in Hz.

- 1 : $\text{R}^1 = \alpha\text{-O-}\beta\text{-D-glucopyranosyl}$, $\beta\text{-H}$ (S); $\text{R}^2 = \text{H}$
 2 : $\text{R}^1 = \alpha\text{-OH}$, $\beta\text{-H}$ (S); $\text{R}^2 = \beta\text{-D-glucopyranosyl}$
 3 : $\text{R}^1 = \alpha\text{-OH}$, $\beta\text{-H}$ (S); $\text{R}^2 = \text{H}$
 4 : $\text{R}^1 = \text{O}$; $\text{R}^2 = \text{H}$
 5 : $\text{R}^1 = \text{NNHC}_6\text{H}_3(\text{NO}_2)_2$; $\text{R}^2 = \text{H}$

Chart 1

TABLE II. ^{13}C -NMR (100.5 MHz) Data for **1**–**4** (δ_{C} , ppm from TMS in $\text{Py}-d_5$)^{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 ^1H - ^{13}C COSY experiments. b) Data for the D-glucosyl moiety 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').

in the formula **3** or its antipode, except for the configuration of the 9-OH group on the side chain. The identification of D-glucose was carried out by paper partition chromatography (PPC), high-performance thin layer chromatography (HPTLC), and gas liquid chromatography (GLC).

The whole relative structure of **3** was determined by a combination of the following chemical study and X-ray analysis. During the course of the chemical derivations of **1**, **2**, and **3**, a novel type of selective bio-oxidation was found. When incubated with Molsin (protease type XIII from *Aspergillus saitoi*),⁶⁾ the ionone (=triol) (**3**) gave the corresponding 3-keto derivative (**4**), $\text{C}_{13}\text{H}_{24}\text{O}_3$, a colorless oil, $[\alpha]_{\text{D}}^{20} + 24.6^\circ$ in 84% yield as a result of a novel selective bio-oxidation reaction, in which only the secondary axial 3-OH group among the primary and two secondary hydroxyls in **3** was oxidized. In a similar treatment with Molsin, both glucosides **1** and **2** also afforded the oxidized aglycone (**4**) (61 and 78% yield, respectively), presumably by usual hydrolysis followed by the selective oxidation.

As the next step, X-ray analysis was undertaken. The oily ketone (**4**) was, in the usual manner, transformed into the corresponding crystalline 2,4-dinitrophenylhydrazone (**5**), $\text{C}_{19}\text{H}_{28}\text{N}_4\text{O}_6$. Crystals of **5** were grown in ethyl acetate as fine red plates of mp 148–150 °C and subjected to a single crystal X-ray analysis. A crystal with dimensions of $0.40 \times 0.15 \times 0.05$ mm was used for collection of X-ray diffraction data at 295 K on an automatic four circle diffractometer equipped with an X-ray tube ($\text{Cu K}\alpha$, $\lambda = 1.5418 \text{ \AA}$) and a graphite monochromator. The cell parameters were determined by least-squares fitting with 20 reflections in the range of $28^\circ < 2\theta < 40^\circ$. The crystal data are as follows: $M_r = 408.3$; monoclinic, space group $P2_1$, $Z = 2$, unit cell dimensions; $a = 15.613(3)$, $b = 8.149(1)$, $c = 8.141(1) \text{ \AA}$, $\beta = 91.32(2)^\circ$, $V = 1035.5(3) \text{ \AA}^3$, $D_m = 1.29(1)$ by flotation on aqueous potassium citrate solution, $D_x = 1.309 \text{ Mg} \cdot \text{m}^{-3}$; $\mu = 8.31 \text{ cm}^{-1}$ for $\text{Cu K}\alpha$ radiation; $F(000) = 436$. The intensities of 1859 unique reflections were obtained within the 2θ range of 125° , in the ω - 2θ scan mode with a scan speed of $\omega = 6^\circ/\text{min}$. The structure was solved by the direct method, SAYTAN87.⁷⁾ All 28 hydrogen atoms were found in the difference Fourier map at the estimated

Fig. 1

positions. Refinement of the structure by the block-diagonal least-squares method converged R to 0.061 for 1504 reflections with $|F_o| = 3\sigma(F_o)$, applying unit weight for each F_{obs} , anisotropic temperature factors for non-hydrogen atoms, and isotropic ones for hydrogen atoms. The atomic scattering factors were taken from International Tables for X-Ray Crystallography.⁸⁾

The right-hand diagram in Fig. 1 shows a perspective view (ORTEP⁹⁾) of the molecular structure of **5**, including an ionone framework with (5*S*, 6*R*, 9*R*) or (5*R*, 6*S*, 9*S*) configuration, and the left hand one shows the numbering system of the molecule **5** used in the present X-ray study. The final atomic parameters are listed in Table III and the bond lengths and angles in Table IV.

Figure 2 shows the presence of three kinds of hydrogen bonds, i.e., an intramolecular one between N17 and O25 (N17---O25 = 2.619(6) Å) and two intermolecular bonds between O11 and O28 (O11---O28 = 2.899(7) Å) and between O15 and O11 (O15---O11 = 2.805 (7) Å) in the crystal structure. The 2,4-dinitrophenylhydrazone part in molecule **5** appears to play an important role in the ready crystallization of **5**, which must be supported by the above-mentioned intermolecular hydrogen bonds (see Fig. 2) and van der Waals contacts [see Fig. 3 (PLUTO¹⁰⁾] observed in the crystal structure of **5**.

Based on this X-ray analysis results, 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 circular dichroism (CD) Cotton effect ($[\theta] + 310$) at 294 nm ($c = 0.083$, EtOH).¹¹⁾ The CD behavior of the ketone (**4**), $[\theta] + 276$ and 294 nm ($c = 0.086$, EtOH) was in agreement with that of nigakialcohol, and this finding indicates that the ketone has the absolute structure shown in the formula **4**. Furthermore, the accumulated lines of evidence demonstrated that the common aglycone is the ionone (**3**) with (3*S*, 5*S*, 6*R*, 9*R*) chiral centers.

As the final step of the structural elucidation, the location of the β -D-glucosyl residue on the aglycone (**3**) was confirmed in both glucosides, **1** and **2**. In the ^{13}C -NMR

TABLE III. Final Atomic Coordinates and Isotropic Temperature Factors of **5** with e.s.d.'s in Parentheses

Atom	x	y	z	B_{eq} or B
C1	1.1061 (3)	0.0844 (9)	-0.3193 (7)	3.20 (6)
C2	1.0371 (4)	-0.0161 (9)	-0.2299 (8)	3.53 (6)
C3	1.0185 (3)	0.0562 (9)	-0.0665 (6)	2.96 (6)
C4	1.0964 (4)	0.0735 (10)	0.0466 (7)	3.64 (7)
C5	1.1692 (4)	0.1676 (9)	-0.0367 (7)	3.35 (6)
C6	1.1882 (3)	0.0974 (9)	-0.2088 (6)	2.93 (6)
C7	1.2610 (3)	0.1920 (9)	-0.2958 (7)	3.38 (7)
C8	1.3390 (4)	0.0840 (11)	-0.3172 (8)	4.32 (7)
C9	1.4100 (3)	0.1586 (11)	-0.4195 (7)	4.21 (9)
C10	1.4422 (4)	0.3233 (11)	-0.3579 (9)	5.02 (9)
O11	1.3786 (3)	0.1693 (10)	-0.5851 (5)	6.73 (10)
C12	1.0688 (4)	0.2539 (10)	-0.3635 (8)	4.30 (7)
C13	1.1265 (4)	-0.0110 (12)	-0.4782 (8)	5.10 (9)
C14	1.2496 (4)	0.1622 (12)	0.0755 (7)	4.73 (10)
O15	1.2308 (3)	0.2392 (9)	0.2246 (6)	5.66 (7)
N16	0.9468 (3)	0.1048 (8)	-0.0089 (5)	3.32 (5)
N17	0.8772 (3)	0.0927 (8)	-0.1186 (5)	3.50 (6)
C18	0.7973 (3)	0.1156 (8)	-0.0619 (6)	2.93 (6)
C19	0.7235 (3)	0.1263 (9)	-0.1662 (6)	3.16 (7)
C20	0.6422 (4)	0.1484 (10)	-0.1086 (7)	3.96 (8)
C21	0.6327 (3)	0.1634 (10)	0.0561 (7)	3.69 (7)
C22	0.7022 (3)	0.1568 (8)	0.1667 (7)	3.08 (6)
C23	0.7825 (4)	0.1332 (9)	0.1080 (6)	3.18 (6)
N24	0.7302 (3)	0.1179 (9)	-0.3453 (6)	4.60 (7)
O25	0.8003 (3)	0.0907 (8)	-0.4065 (5)	5.13 (7)
O26	0.6653 (3)	0.1372 (12)	-0.4284 (5)	8.40 (13)
N27	0.5473 (3)	0.1892 (11)	0.1215 (7)	5.68 (10)
O28	0.5411 (3)	0.2293 (9)	0.2636 (6)	6.01 (8)
O29	0.4855 (3)	0.1603 (13)	0.0314 (6)	9.37 (16)
H1	0.981 (4)	-0.026 (10)	-0.303 (8)	3.4
H2	1.058 (4)	-0.131 (10)	-0.207 (8)	3.4
H3	1.119 (4)	-0.047 (10)	0.077 (8)	3.7
H4	1.077 (4)	0.137 (10)	0.156 (8)	3.7
H5	1.152 (4)	0.295 (10)	-0.053 (8)	3.4
H6	1.209 (4)	-0.028 (10)	-0.190 (8)	3.1
H7	1.278 (4)	0.298 (10)	-0.224 (8)	3.6
H8	1.240 (4)	0.233 (10)	-0.415 (8)	3.6
H9	1.320 (5)	-0.028 (11)	-0.378 (9)	4.5
H10	1.363 (5)	0.055 (11)	-0.198 (9)	4.4
H11	1.467 (5)	0.083 (12)	-0.412 (8)	4.8
H12	1.493 (5)	0.366 (11)	-0.438 (9)	4.9
H13	1.466 (5)	0.308 (11)	-0.235 (9)	4.9
H14	1.391 (5)	0.410 (11)	-0.354 (9)	4.9
H15	1.421 (5)	0.218 (13)	-0.655 (10)	7.0
H16	1.117 (5)	0.325 (11)	-0.427 (9)	4.6
H17	1.048 (5)	0.316 (11)	-0.253 (9)	4.5
H18	1.014 (5)	0.237 (11)	-0.441 (8)	4.6
H19	1.175 (5)	0.059 (11)	-0.550 (9)	5.0
H20	1.069 (5)	-0.015 (11)	-0.552 (9)	5.0
H21	1.148 (5)	-0.129 (11)	-0.454 (9)	5.0
H22	1.303 (5)	0.223 (11)	0.014 (9)	5.2
H23	1.267 (5)	0.036 (11)	0.097 (9)	5.1
H24	1.280 (5)	0.235 (12)	0.292 (9)	5.4
H25	0.888 (4)	0.053 (10)	-0.234 (8)	3.4
H26	0.588 (4)	0.154 (11)	-0.192 (8)	4.2
H27	0.690 (4)	0.166 (9)	0.297 (7)	3.1
H28	0.835 (4)	0.130 (10)	0.191 (7)	3.2

$$B_{eq} = (4/3) \sum_i \sum_j B_{ij} a_i a_j \text{ for non-hydrogen atoms.}$$

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 of **3**. On the other hand, C-9 and both C-8 and C-10 of **2** were respectively shifted downfield (C-9; +8.67 ppm) and up-field (C-8 and C-10; -3.64 and -2.58 ppm, respectively)

TABLE IV. Bond Lengths (Å) and Angles (°) for non Hydrogen Atoms of **5** with e.s.d.'s in Parentheses

Bond lengths:			
C1-C2	1.554 (11)	C1-C6	1.555 (11)
C1-C13	1.554 (13)	C2-C3	1.487 (11)
C3-N16	1.286 (10)	C4-C5	1.538 (11)
C5-C14	1.536 (13)	C6-C7	1.565 (11)
C8-C9	1.525 (12)	C9-C10	1.501 (13)
C14-O15	1.407 (12)	N16-N17	1.338 (10)
C18-C19	1.417 (10)	C18-C23	1.414 (10)
C19-N24	1.461 (11)	C20-C21	1.358 (12)
C21-N27	1.462 (12)	C22-C23	1.362 (10)
N24-O26	1.217 (13)	N27-O28	1.210 (12)
N27-O29	1.217 (14)		
Bond angles:			
C2-C1-C6	109.5 (6)	C2-C1-C12	108.7 (6)
C2-C1-C13	107.5 (7)	C6-C1-C12	112.5 (7)
C6-C1-C13	110.1 (7)	C12-C1-C13	108.3 (7)
C1-C2-C3	111.0 (6)	C2-C3-C4	114.0 (6)
C2-C3-N16	129.2 (7)	C4-C3-N16	116.8 (7)
C3-C4-C5	112.0 (7)	C4-C5-C6	111.5 (6)
C4-C5-C14	109.0 (7)	C6-C5-C14	110.7 (7)
C1-C6-C5	112.0 (6)	C1-C6-C7	111.6 (6)
C5-C6-C7	112.5 (6)	C6-C7-C8	111.0 (6)
C7-C8-C9	115.1 (7)	C8-C9-C10	114.9 (8)
C8-C9-O11	107.0 (7)	C10-C9-O11	111.5 (8)
C5-C14-O15	108.5 (8)	C3-N16-N17	115.1 (6)
N16-N17-C18	119.2 (6)	N17-C18-C19	123.1 (7)
N17-C18-C23	121.2 (7)	C19-C18-C23	115.8 (6)
C18-C19-C20	122.9 (7)	C18-C19-N24	121.4 (7)
C20-C19-N24	115.7 (7)	C19-C20-C21	118.4 (8)
C20-C21-C22	121.7 (8)	C20-C21-N27	119.7 (8)
C22-C21-N27	118.6 (7)	C21-C22-C23	119.6 (7)
C18-C23-C22	121.6 (7)	C19-N24-O25	119.7 (7)
C19-N24-O26	118.2 (8)	O25-N24-O26	122.1 (9)
C21-N27-O28	118.8 (8)	C21-N27-O29	118.1 (9)
O28-N27-O29	123.2 (9)

Fig. 2

Fig. 3

compared with those for **3**. In addition, in the NOESY spectra of **1** and **2**, there were typical cross peaks between 3β -H and the glucosyl anomeric proton in **1** and between 9-H and the glucosyl anomeric proton in **2**. These NMR studies proved that the β -D-glucosyl moiety is linked at 3α -OH of the aglycone (**3**) in **1** and at 9-OH of the aglycone in **2**. Based on the accumulated evidence, the absolute structures of melia-ionosides A and B are defined as **1** and **2**, respectively.

This is the first time that **1** and **2** have been found in nature, and their common aglycone (**3**) is a new saturated ionone.

Experimental

All melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. The spectral data were obtained on the following instruments: optical rotation on a JASCO DIP-140, IR on a JASCO A-302, ^1H - and ^{13}C -NMR on a JEOL GX-400, EI- and accurate MS (ionization voltage; unless otherwise stated, 30 eV) and negative ion FAB-MS (matrix; triethanolamine) on a JEOL JNM-DX300, and CD on a JASCO J-500C. GLC was carried out on a Shimadzu GC-7AG under the following conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m $\times$ 3 mm i.d.); detector, hydrogen flame ionization detector; column temperature, 160 $^\circ\text{C}$; carrier N_2 gas, 30 ml/min. As silica gel for column chromatography, Kieselgel 60 (Merck, 230–400 mesh) was used. Preparative HPLC was carried out on a Kusano instrument (Kusano Scientific Co., Tokyo) with a KPW-10 micro pump and a Shodex SE-31 differential refractometer under the following conditions: column, a reversed-phase Kusano octadecyl silica (ODS) (10 cm $\times$ 22 mm i.d.) and a Kusano Si-10 prepacked column (10 cm $\times$ 22 mm i.d.); flow rate, 3 ml/min. In the purification of the 2,4-dinitrophenylhydrazone (**5**), a Waters HPLC system [an M45J pump, an R-401 differential refractometer and a silica-phase μ -Porasil column (30 cm $\times$ 7.8 mm i.d.)] was used, with an eluant flow of 3 ml/min of *n*-hexane–AcOEt (1:3). The X-ray intensity data were measured on a Rigaku AFC-5 automatic diffractometer at room temperature. The Molsin (protease type XIII from *Aspergillus saitoi*) used in this work was a commercial product (Sigma Chem. Co., Lot. No. 104F-0124).

Plant Material and Extraction The same procedure as mentioned in

refs. 3 and 4 was employed.

Isolation of Glucosides 1 and 2 The MeOH extract (287 g) obtained from the air-dried leaves of *M. toosendan* was suspended in H_2O and the suspension was extracted successively with petroleum ether (500 ml $\times$ 3), CHCl_3 (500 ml $\times$ 4), and *n*-BuOH (400 ml $\times$ 2). The residue (52.4 g) obtained from the *n*-BuOH layer was subjected to silica gel column chromatography [1 kg; eluted with CHCl_3 –MeOH– H_2O (35:20:4)] and subsequent Sephadex LH-20 column chromatography (100 g; eluted with MeOH) to afford a fraction (2.87 g) containing **1**, **2**, and toosendanoside.⁴⁾ Successive HPLC separation of the fraction on ODS [eluent, MeOH– H_2O (1:1)] and Si-10 [eluent, CHCl_3 –MeOH (6:1)] columns gave pure toosendanoside and a mixture (138 mg) containing **1** and **2**. This mixture was further purified by ODS HPLC separation [eluent, MeOH– H_2O (5:6)] to furnish pure glucosides **1** (21 mg) and **2** (9.5 mg), respectively.

Melia-ionoside A (1): A white powder (from a mixture of a large amount of C_6H_6 and a few drops of MeOH), $[\alpha]_{\text{D}}^{20} -35.1^\circ$ ($c=0.43$, py.). IR (KBr) cm^{-1} : 3350, 1075. Negative ion FAB-MS m/z [%]: 391 [(M–H) $^-$, 100]. EI-MS m/z (%): 213 ($\text{M}^+ + \text{H} - 162 - \text{H}_2\text{O}$, 5), 195 ($\text{M}^+ + \text{H} - 162 - 2\text{H}_2\text{O}$, 30), 177 ($\text{M}^+ + \text{H} - 162 - 3\text{H}_2\text{O}$, 100). ^1H - and ^{13}C -NMR: see Tables I and II, respectively. Anal. Calcd for $\text{C}_{19}\text{H}_{36}\text{O}_8$: C, 58.14; H, 9.25. Found: C, 58.13; H, 9.22.

Melia-ionoside B (2): A white powder (from the same solvent mixture as in the case of **1**), $[\alpha]_{\text{D}}^{20} -9.0^\circ$ ($c=0.15$, py.). IR (KBr) cm^{-1} : 3350, 1075. Negative ion FAB-MS m/z [%]: 391 [(M–H) $^-$, 100]. EI-MS m/z (%): 355 ($\text{M}^+ - \text{H} - 2\text{H}_2\text{O}$, 4), 231 ($\text{M}^+ + \text{H} - 162$, 8), 213 ($\text{M}^+ + \text{H} - 162 - \text{H}_2\text{O}$, 43), 195 ($\text{M}^+ + \text{H} - 162 - 2\text{H}_2\text{O}$, 69), 177 ($\text{M}^+ + \text{H} - 162 - 3\text{H}_2\text{O}$, 100). ^1H - and ^{13}C -NMR: see Tables I and II, respectively. Anal. Calcd for $\text{C}_{19}\text{H}_{36}\text{O}_8 \cdot 1/2\text{H}_2\text{O}$: C, 56.84; H, 9.29. Found: C, 57.05; H, 9.04.

Acidic Hydrolysis of 1 A solution of **1** (26 mg) in MeOH (6 ml) and 10% H_2SO_4 (3 ml) was refluxed for 4 h, poured into ice-water, and extracted with AcOEt (60 ml $\times$ 1 and 40 ml $\times$ 3) and *n*-BuOH (60 ml $\times$ 2), successively. Each of the AcOEt and *n*-BuOH extracts was washed with 5% aqueous NaHCO_3 and then H_2O , and dried over MgSO_4 . The AcOEt and *n*-BuOH extracts were combined and evaporated to dryness. The residual product was purified by silica gel column chromatography [eluent, CHCl_3 –MeOH (10:1) to give an aglycone (**3**) (12 mg, 78.4% yield), colorless oil, $[\alpha]_{\text{D}}^{20} -6.8^\circ$ ($c=0.10$, py.). IR (CHCl_3) cm^{-1} : 3605, 3400. EI- and accurate MS (ionization voltage, at 20 eV) m/z (%): 231.195 ($\text{M}^+ + \text{H}$, Calcd for $\text{C}_{13}\text{H}_{27}\text{O}_3$ 231.196, 3), 230.188 (M^+ , Calcd for $\text{C}_{13}\text{H}_{26}\text{O}_3$ 230.188, 1), 212.178 ($\text{M}^+ - \text{H}_2\text{O}$, Calcd for $\text{C}_{13}\text{H}_{24}\text{O}_2$ 212.178, 6), 194.168 ($\text{M}^+ - 2\text{H}_2\text{O}$, Calcd for $\text{C}_{13}\text{H}_{22}\text{O}$ 194.167, 39), 176 ($\text{M}^+ - 3\text{H}_2\text{O}$, 23), 122 (100). Negative ion FAB-MS m/z [%]: 229 [(M–H) $^-$, 30]. ^1H - and ^{13}C -NMR: see Tables I and II, respectively. The aqueous layer of the hydrolysate was neutralized with Amberlite IRA-45 (OH^- form) and evaporated under reduced pressure. The residual product was subjected to PPC [iso-PrOH–*n*-BuOH– H_2O (7:1:2) as the developing solvents; developed twice; aniline hydrogen phthalate for detection], HPTLC [Merck Si 50000 type; *n*-BuOH–py.– H_2O (75:15:10) as the developing solvents], and GLC [after trimethylsilylation with *N,O*-bis(trimethylsilyl)-trifluoroacetamide–py.] to demonstrate the presence of glucose.

Acidic Hydrolysis of 2 A solution of **2** (60 mg) in 10% H_2SO_4 (5 ml) and MeOH (10 ml) was refluxed for 4 h. In the same manner as in the case of acidic hydrolysis of **1**, the reaction mixture was worked up. The aglycone **3** (25.4 mg, 72.2% yield) and glucose were isolated and identified.

Enzymatic Hydrolysis of 1 A suspension of Molsin (protease type XIII from *Aspergillus saitoi*) (400 mg) in 0.2 M citric acid–0.2 M Na_2HPO_4 buffer (pH 4.0; 3 ml) was added to a solution of **1** (13 mg) in EtOH (0.4 ml). The reaction mixture was stirred at 37 $^\circ\text{C}$ for 63 h, then poured into H_2O , and extracted with AcOEt (60 ml $\times$ 1 and 40 ml $\times$ 3) and *n*-BuOH (50 ml $\times$ 2), successively. The AcOEt and *n*-BuOH extracts were each washed with H_2O , and dried over MgSO_4 . The two extracts were combined and evaporated to dryness. The residue was purified by silica gel column chromatography [eluent, CHCl_3 –MeOH (7:1)] to give the corresponding 3-keto derivative (**4**) (4.6 mg, 61% yield) of the aglycone (**3**), together with a small amount of **3** (1 mg, 13% yield). The physical and spectral properties of **4** are as follows: colorless oil, $[\alpha]_{\text{D}}^{20} +11.7^\circ$ ($c=0.185$, py.) and $[\alpha]_{\text{D}}^{20} +24.6^\circ$ ($c=0.35$, CHCl_3). IR (CHCl_3) cm^{-1} : 3620, 3410 (OH), 1700 (C=O). EI- and accurate MS m/z (%): 228.172 (M^+ , Calcd for $\text{C}_{13}\text{H}_{24}\text{O}_3$ 228.173, 1), 213.149 ($\text{M}^+ - \text{CH}_3$, Calcd for $\text{C}_{12}\text{H}_{21}\text{O}_3$ 213.149, 14), 210.162 ($\text{M}^+ - \text{H}_2\text{O}$, Calcd for $\text{C}_{13}\text{H}_{22}\text{O}_2$ 210.162, 25), 195.139 ($\text{M}^+ - \text{H}_2\text{O} - \text{CH}_3$, Calcd for $\text{C}_{12}\text{H}_{19}\text{O}_2$ 195.139, 23), 192.151 ($\text{M}^+ - 2\text{H}_2\text{O}$, Calcd for $\text{C}_{13}\text{H}_{20}\text{O}$ 192.151, 6), 177.128 ($\text{M}^+ - 2\text{H}_2\text{O} - \text{CH}_3$, Calcd for $\text{C}_{12}\text{H}_{17}\text{O}$ 177.128, 35), 83 (100). Negative ion FAB-MS m/z [%]: 227 [(M–H) $^-$, 45]. ^1H -NMR (py.- d_5) δ : 0.80 (3H, s, 11- H_3), 1.04 (3H, s, 12- H_3), 1.38 (3H, d, $J=6.1$ Hz,

10-H₃), 1.6 (1H, m, 7-H), 1.7 (3H, m, 6 β -H, 7-H, and 8-H), 1.93 (1H, m, $W_{1/2}$ = 18.0 Hz, 5 α -H), 2.02 (1H, m, 8-H), 2.15 (1H, dd, J = 13.1, 2.1 Hz, 2 α -H), 2.36 (1H, d, J = 13.1 Hz, 2 β -H), 2.70 (1H, ddd, J = 14.0, 5.2, 2.1 Hz, 4 α -H), 2.76 (1H, dd, J = 14.0, 11.9, 4 β -H), 4.0 (1H, m, 9-H), 3.92 (1H, dd, J = 10.7, 2.8 Hz), 4.01 (1H, dd, J = 10.7, 4.9 Hz) (13-H₂). ¹³C-NMR: see Table II. CD (c = 0.086, EtOH) $[\theta]$ (nm): +276 (294) (positive maximum) and (c = 0.050, CHCl₃) $[\theta]$ (nm): +336 (287) (positive maximum).

Enzymatic Hydrolysis of 2 A suspension of Molsin (700 mg) in 0.2 M citric acid–0.2 M Na₂HPO₄ buffer (pH 4.0, 5 ml) was added to a solution of **2** (21.0 mg) in EtOH (0.8 ml). The mixture was incubated with stirring at 37 °C for 120 h, then worked up and purified in the same manner as in the case of enzymic hydrolysis of **1**. The 3-keto derivative (**4**) (9.5 mg, 78% yield) was obtained as a sole product.

Bio-oxidation of 3 A suspension of Molsin (120 mg) in 0.2 M citric acid–0.2 M Na₂HPO₄ buffer (pH 4.0, 3 ml) was added to a solution of **3** (8.2 mg) in EtOH (0.3 ml). The mixture was allowed to react under stirring at 37 °C for 96 h, and worked up and purified in the same manner as described for the enzymic hydrolysis of **1** and **2**. The corresponding 3-keto derivative (**4**) (6.8 mg) was obtained in high yield (84%) as a sole reaction product.

Conversion of 4 to the Corresponding 2,4-Dinitrophenylhydrazone (5) A 0.5 ml aliquot of a 2,4-dinitrophenylhydrazine solution (0.07 M), prepared by adding concentrated H₂SO₄ (0.2 ml), H₂O (0.3 ml), and 95% aqueous EtOH (1 ml) successively to 2,4-dinitrophenylhydrazine (0.04 g), was added dropwise to a solution of **4** (4.9 mg, 0.021 mmol). The reaction mixture was allowed to stand for 2 d at room temperature, poured into ice-H₂O, and extracted with AcOEt (30 ml $\times$ 4). The combined AcOEt layer was washed with aqueous NaHCO₃ and H₂O successively, dried over MgSO₄, and evaporated to dryness. The residue was subjected to purification by a Waters HPLC system to give the corresponding 2,4-dinitrophenylhydrazone (**5**) (3.0 mg) of **4**. Recrystallization from AcOEt afforded red plates of mp 148–150 °C. EI-MS m/z (%): 408 (M⁺, 50), 390 (M⁺ – H₂O, 39), 81 (100). ¹H-NMR (acetone-*d*₆) δ : 0.82 (3H, s, 11-H₃), 1.14 (3H, d, J = 6.1, 10-H₃), 1.19 (3H, s, 12-H₃), 1.4 (1H, m, 6 β -H), 1.5 (3H, m, 7-H₂ and 8-H), 1.6 (1H, m, 8-H), 1.74 (1H, m, 5 α -H), 2.16 (1H, d, J = 13.7 Hz, 2 β -H), 2.38 (1H, dd, J = 13.4, 12.8 Hz, 4 β -H), 2.65 (1H, dd, J = 13.7, 2.4 Hz, 2 α -H), 2.71 (1H, ddd, J = 13.4, 4.6, 2.4 Hz, 4 α -H), 3.71 (1H, m, 9-H), 3.64 (1H, dd, J = 11.2, 6.6 Hz), 3.77 (1H, dd, J = 11.2, 3.4 Hz) (13-H₂), 8.04 (1H, d, J = 9.5 Hz, aromatic 6-H), 8.38 (1H, dd, J = 9.5, 2.7 Hz, aromatic 5-H), 9.00 (1H, d, J = 2.7 Hz, aromatic 3-H), 11.22 (1H, s, –NH–).

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References and Notes

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