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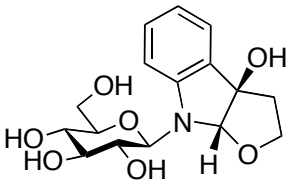

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**Abstract**

Makomotindoline (**1**) was isolated from Makomotake, *Zizania latifolia* infected with *Ustilago esculenta*. The structure was determined by the interpretation of spectroscopic data and synthesis. Makomotindoline (**1**), its L-Glc isomer (**2**) and its aglycon (**3**) were synthesized and their effects on rat glioma cells showed adverse effects on the cell growth.

*Keywords*: Makomotake, *Ustilago esculenta*, structure determination, glioma cell, synthesis

The fungus, *Ustilago esculenta*, penetrates into the aquatic perennial grass, *Zizania latifolia*. After penetration, the fungus incites the formation of an edible gall and inhibits inflorescence and seed production in the plant. The gall is called Makomotake in Japanese and Jiaobai in Chinese, and an edible product in Japan, China and other Asian countries. We have already reported osteoclast-forming suppressive nortriterpenes from it[1] and in the course of further search for new compounds from Makomotake, we succeeded in purifying a novel *N*-glucoside. Here we describe the isolation, structure determination, synthesis and biological activity of the compound and its analogues.

Fresh Makomotake was extracted with EtOH and then with acetone. After the solutions had been combined and concentrated under reduced pressure, the concentrate was partitioned between $CH_2Cl_2$ and $H_2O$, and then the $H_2O$ soluble part was partitioned between EtOAc and $H_2O$. The EtOAc soluble part was fractionated by repeated chromatography, resulting in compound **1**.

Makomotindoline (**1**)[2] was purified as a colorless amorphous solid. Its molecular formula was determined as $C_{16}H_{21}NO_7$ by HRESIMS *m/z* 362.1191 $[M+Na]^+$ (calcd for $C_{16}H_{21}NO_7Na$, 362.1216),[3] indicating the presence of seven degrees of unsaturation in the molecule. The structure of **1** was elucidated by interpretation of NMR spectra[3]

including DEPT, COSY, HMQC, and HMBC (Fig. 1). The complete assignment of the protons and carbons was accomplished as shown in Table 1. The presence of the β-glucosyl group was suggested by the characteristic chemical shifts and coupling constants: $\delta_C$ 86.2 (C-1'), 71.3 (C-2'), 77.0 (C-3'), 69.8 (C-4'), 77.9 (C-5'), 61.0 (C-6'); $\delta_H$ 4.88 (d, *J* = 9.2 Hz, H-1'), 3.68 (dd, *J* = 9.2, 9.5 Hz, H-2'), 3.53 (dd, *J* = 9.5, 8.9 Hz, H-3'), 3.39 (dd, Hz, *J* = 8.9, 9.5 Hz, H-4'), 3.42 (m, H-5'), 3.61 (dd, *J* = 12.2, 4.8 Hz, H-6a'), 3.73 (dd, *J* = 12.2, 1.6 Hz, H-6b'). The presence of a 1,2-substituted phenyl group (C-3b to C-7a) in the aglycon was also suggested by the NMR data: $\delta_C$ 131.2 (C-3b), 124.9 (C-4), 121.6 (C-5), 130.9 (C-6), 109.6 (C-7), 148.5 (C-7a); $\delta_H$ 7.33 (d, *J* = 7.3 Hz, H-4), 6.92 (dd, *J* = 7.3, 7.3 Hz, H-5), 7.25 (dd *J* = 7.3, 7.6 Hz, H-6), 6.83 (d, *J* = 7.6 Hz, H-7). The structure of the other part, the condensed ring part, in the aglycon and the linkage position with the phenyl were elucidated by the correlations in the HMBC data (Fig. 1) and the molecular formula. The *N*-glucosidic bond and its position between the sugar and the aglycon were determined by the HMBC correlations (H-8a/H-1', H-1'/H-7a, H-1'/H-8a, Fig. 1) and the relatively high field chemical shift at C-1' ($\delta_C$ 86.2) compared with those of

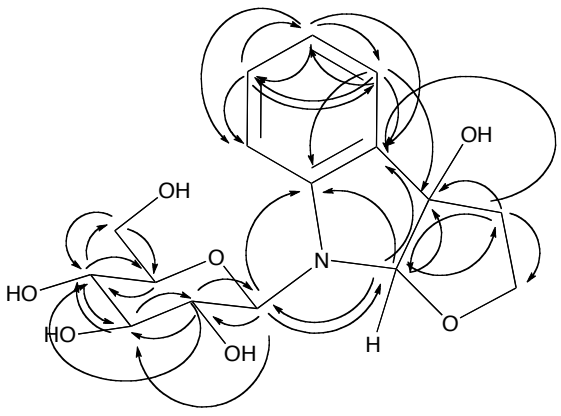
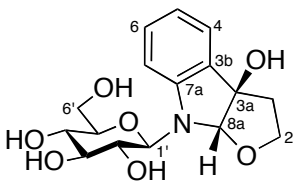



**Figure 1.** HMBC correlations in **1**

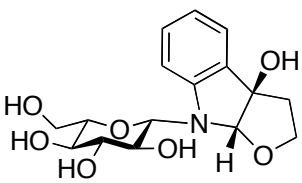
*O*-glycosides.[4, 5]

**Table 1**
$^{1}H$ and $^{13}C$ NMR data for **1** and **2** (in $D_2O$)

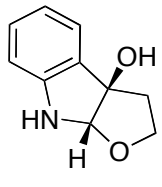
| Position | **1** | | **2** | |
|---|---|---|---|---|
| | $^{1}H$ δ (multiplicity, *J* in Hz) | $^{13}C$ δ | $^{1}H$ δ (multiplicity, *J* in Hz) | $^{13}C$ δ |
| Aglycone | | | | |
| 2 | 3.57 (m) | 67.8 | 3.59 (m) | 68.3 |
| | 4.02 (m) | | 4.05 (m) | |
| 3 | 2.33 (m) | 40.6 | 2.45 (m) | 39.9 |
| 3a | | 87.8 | | 87.8 |
| 3b | | 131.2 | | 130.2 |
| 4 | 7.33 (d, 7.3) | 124.9 | 7.39 (dd, 7.6, 0.9) | 125.3 |
| 5 | 6.92 (dd, 7.3, 7.3) | 121.6 | 6.93 (dd, 7,6, 7.3) | 121.1 |
| 6 | 7.25 (dd 7.3, 7.6) | 130.9 | 7.29 (ddd, 7.3, 7.9, 0.9) | 131.3 |
| 7 | 6.83 (d, 7.6) | 109.6 | 6.78 (d, 7.9) | 108.2 |
| 7a | | 148.5 | | 148.7 |
| 8a | 5.59 (s) | 99.7 | 5.51 (s) | 98.4 |
| Sugar | | | | |
| 1’ | 4.88 (d, 9.2) | 86.2 | 4.89 (d, 9.2) | 85.5 |
| 2’ | 3.68 (dd, 9.2, 9.5) | 71.3 | 3.78 (dd, 9.2, 8.9) | 70.1 |
| 3’ | 3.53 (dd, 9.5, 8.9) | 77.0 | 3.59 (dd, 8.9, 8.8) | 78.0 |
| 4’ | 3.39 (dd, 8.9, 9.5) | 69.8 | 3.48 (dd, 8.8, 9.8) | 69.9 |
| 5’ | 3.42 (m) | 77.9 | 3.54 (ddd, 9.9, 4.9, 2.1) | 78.0 |
| 6’ | 3.61 (dd, 12.2, 4.8) | 61.0 | 3.73 (dd, 12.5, 4.9) | 61.2 |
| | 3.73 (dd, 12.2, 1.6) | | 3.83 (dd, 12.5, 2.1) | |




**1**



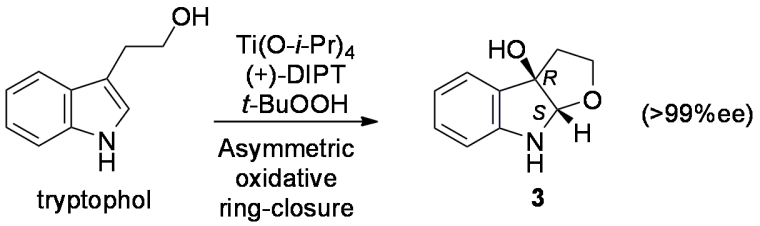

**2**



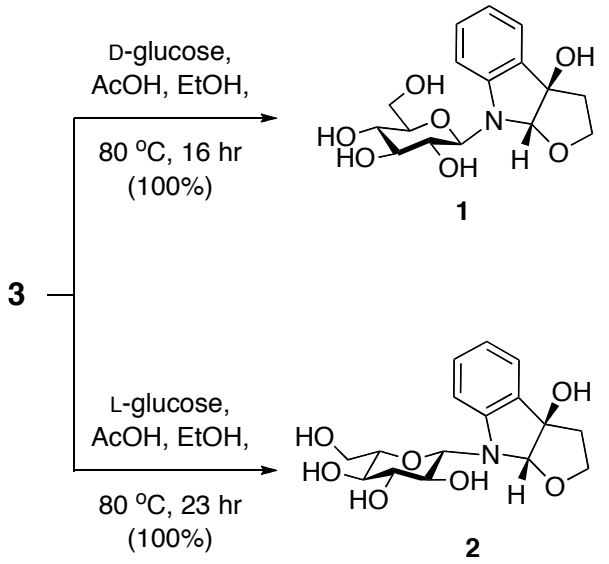

**3**

To confirm the planar structure and determine the whole absolute configuration of **1**, an optically active D-glucoside and L-glucoside, **1** and **2**,[6]were synthesized

chemically. Previously, we completed total synthesis of madindoline A, involving an asymmetric oxidative ring-closure reaction of tryptophol to give **3** by modified Sharpless epoxidation [Ti(O-*i*-Pr)$_4$, (+)-DIPT, *t*-BuOOH, $CH_2Cl_2$, –20 to –40°C (Scheme 1).[7,8] Interest in the synthetic applications of **3** led us to develop to the asymmetric total synthesis of other optically active indole alkaloid, (–)-physovenine,[9] in a concise manner.[10] Two diastereomers, **1** and **2**, were prepared by the *N*-glucosidation of **3** with D- and L-glucoses[11,12,13] in quantitative yields, respectively (Scheme 2). The NMR data and the specific rotation ($[\alpha]_D^{21}$ –45, $H_2O$, *c* 0.07)[3] of synthetic **1** were completely identical with those of natural **1**, indicating that the absolute configuration of **1** was as shown.




Scheme 1




**Scheme 2**

The effects of the compounds on C6 cell viability were analyzed.[14] The natural

product (**1**) showed no effect. However, 100 μM of the aglycone (**3**) significantly stimulated the cell growth at 24 h (112.0 ± 9.8%) and 48 h (114.2 ± 11.0%) treatment. On the other hand, L-glucoside (**2**) significantly reduced the cell number at 10 μM (92.4 ± 9.1%) and 100 μM (83.9 ± 8.0%) in 24 h treated cells, and at 100 μM (87.9 ± 11.1%) in 48 h treated cells. Since dead cells were hardly observed by the treatment with **2** (data not shown), the reducing effect of the compound is thought to be growth retardation. Glycosylated natural products such as saponins or glycoalkaloids have been reported to show anti-proliferative activity or apoptotic effect in mammalian cells, especially cancer cells.[15] Although they are expected to be utilized as anti-cancer agents, some of them show cytotoxicity even in normal cells. Tomato saponin called as tomatine, has been reported to be cytotoxic, and its deglycosylation abolished the cytotoxicity.[16] It raises the possibility that each compound used in this study show distinct effect on mammalian cells.

**Acknowledgment**

We thank V. K. Deo (Shizuoka University) for valuable discussion.

**References and notes**

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2. The material, Makomotake, was purchased from local market in Shizuoka, Japan. Fresh Makomotake (16.7 kg) was extracted with EtOH (22 L, 3 times) and then with acetone (10 L, once). After the solutions had been combined and concentrated under reduced pressure, the concentrate was partitioned between $CH_2Cl_2$ and $H_2O$, and then the $H_2O$ soluble part was partitioned between EtOAc and $H_2O$. The residue (20.0 g) obtained after removing EtOAc was fractionated by silica gel flash column chromatography (80% $CH_2Cl_2$/acetone, 50% $CH_2Cl_2$/MeOH, MeOH, each 2 L) to obtain seven fractions. Fraction 2 (eluted with 80% $CH_2Cl_2$/acetone, 1.28 g) was further separated by reversed-phase HPLC (Develosil C30-UG, 15% MeOH, 5 mL/tube), and nine fractions were obtained. Fraction 2-5 (65.7 mg) was further fractionated by reversed-phase HPLC (Cosmosil cholester, 10% MeOH) to afford compound **1** (1.8 mg; *Rt* 77.6 min). Compound **1**. mp 154-156°C; $[\alpha]_D^{28}$ – 48 (*c* 0.30, $H_2O$); IR (neat); 1486, 1608, 3367 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; ESIMS *m/z* 362 $[M+Na]^+$; HRESIMS *m/z* 362.1191 $[M+Na]^+$ (calcd for $C_{16}H_{21}NO_7Na$, 362.1216).

3. $^1H$ NMR spectra (one- and two-dimensional) were recorded on a JEOL lambda-500 spectrometer at 500 MHz, while $^{13}C$ NMR spectra were recorded on the same instrument at 125 MHz. The HRESIMS spectra were measured on a JMS-T100LC mass

spectrometer. A JASCO grating infrared spectrophotometer was used to record the IR spectra.

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6. Compound **2**. mp 131-132°C; $[\alpha]_D^{28}$ –12 (*c* 0.30, $H_2O$); IR (neat); 1487, 1608, 3387 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; ESIMS *m/z* 362 $[M+Na]^+$; HRESIMS *m/z* 362.1191 $[M+Na]^+$ (calcd for $C_{16}H_{21}NO_7Na$, 362.1216).

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13. To solution of **3** (9.3 mg, 0.0525 mmol) in dry EtOH (0.53 mL), D-glucose (28.4 mg, 0.157 mmol) and acetic acid (9.0 mL, 0.157 mmol) at room temperature were added and the mixture was warmed up to 80°C with stirring for 16 h. Then, the reaction mixture was cooled to room temperature and concentrated. Flash chromatography ($CHCl_3$/MeOH = 5/1) gave the mixture of desired product and slight amount of D-glucose , which was further purified by Sep-Pak Plus C18 Cartridge ($CH_3CN/H_2O$ = 1/9) to afford **1** (19.9 mg, 100%) as a colorless solid. According to the procedure of **1**, **2** (19.9 mg, 100%) was obtained as a colorless solid from **3** (10.3 mg, 0.0581 mmol) and L-glucose (31.4 mg, 0.174 mmol) with acetic acid (10 μL, 0.174 mmol) in EtOH (0.58 mL).

14. Rat glioma cell line C6 cells were maintained in the Dulbecco's modified Eagles medium (D-MEM, SIGMA, USA) supplemented with 10% fetal bovine serum (FBS), 10 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in humidified 5% $CO_2$ atmosphere. Cell number analysis was performed by 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2H- tetrazolium bromide (MTT) assay. C6 cells were cultured in 96 well plates at 2000 cells /well cell density. After one-day culture, the cells were treated with

serial concentrations of the compounds in the medium without FBS. After 24 or 48 h incubation, the treated cells were subjected to the MTT assay. The treatment medium was replaced to the medium supplemented with 0.25 mg/mL of MTT without FBS, and the cells were incubated for 2 h. The incubation was terminated by addition of 20% (w/v) sodium dodecylsulfate (SDS) and 50% (v/v) dimethylformamide in water. The absorbance at 570 nm of the reaction mixture was measured by a microplate reader (Molecular Devices, USA). The data were expressed as means ± SD. The difference was analyzed by one-way ANOVA followed by Tukey-Kramer post-hoc test. P values less than 0.05 was considered significant.

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