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Locustoside A — A new purine alkaloid glucoside from seeds of *Gleditsia japonica*

Tadashi Kajimoto, Nobuwa Aoki, Emi Ohta, Shinji Ohta *

Nagahama Institute of Bio-Science and Technology, 1266 Tamura-cho, Nagahama, Shiga 526-0829, Japan

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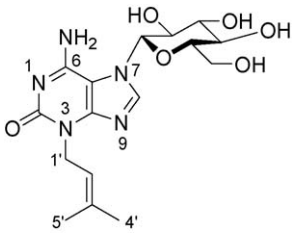
ABSTRACT

A new purine alkaloid glucoside designated locustoside A has been isolated from the seeds of *Gleditsia japonica*, which has been used in oriental traditional medicine. The structure was determined as 7-β-D-glucopyranosyl-3-(3-methyl-2-butenyl)-isoguanine by interpretation of spectroscopic data and was confirmed by X-ray crystallographic analysis.

1. Introduction

Japanese honey locust, *Gleditsia japonica* Miquel (Leguminosae) has been used as a diuretic and an expectorant in oriental traditional medicine (Konoshima et al., 1995). Its seeds have been reported to contain polyamines (Hamana et al., 1996), phenols, alkaloids, flavonoids, carbohydrates, saponins, steroids, coumarins, and amino acids (Wan et al., 2001). During our continuing search for biologically active compounds from plants (Aoki et al., 2008; Kajimoto et al., 2010), we isolated a new purine alkaloid glucoside designated locustoside A (**1**) from the MeOH extract of the seeds of *G. japonica*. This report describes the purification and the structure elucidation of **1**.



1

2. Results and discussion

The seeds (166 g) of *G. japonica* were cut into small pieces and extracted with MeOH. The concentrated MeOH extract was suspended in water and partitioned successively with hexane and EtOAc. The aqueous layer was lyophilized to yield a syrup. The water-soluble fraction (8.4 g) was separated repeatedly on an ODS column employing MeOH in H_2O gradient mixtures to afford **1** (8.8 mg, 0.0053%) as colorless plates, mp 209–212 °C, $[\alpha]_D^{25}$ +35.2 (*c* 0.44, H_2O).

Locustoside A (**1**) exhibited a pseudomolecular ion peak at *m*/*z* 382 corresponding to $[M+H]^+$ in the (+)ESIMS. The molecular formula of **1** was established to be $C_{16}H_{23}N_5O_6$ on the basis of high-resolution ESITOFMS data (*m*/*z* 382.1716 $[M+H]^+$, Δ −1.1 mmu). The IR spectrum displayed absorption bands at 3600–3000 (OH, NH, and CH), 1641, 1616, and 1583 cm^{-1} (C=O, C=N, and C=C). The UV (MeOH) absorption maxima at 246 (log ε 3.86) and 290 nm (log ε 3.67) suggested the presence of a 3,7-disubstituted isoguanine skeleton (Stewart and Harris, 1977; Cafieri et al., 1995), which was supported by the ^1H and ^{13}C NMR chemical shifts [δ_H 8.12 (1H, s, H-8), δ_C 157.6 (C-2), 153.6 (C-4), 104.5 (C-5), 154.5 (C-6), 144.9 (C-8)]. Inspection of the ^1H and ^{13}C NMR spectra (Table 1) together with the DEPT and HMQC spectral data revealed the presence of two olefinic methyls, two hetero atom bearing methylenes, five oxygenated methines, an olefinic methine, and a quaternary olefinic carbon in addition to the isoguanine unit. The presence of an isopentenyl unit was suggested by the observation of a fragment ion peak at *m*/*z* 314 $[M-67]^+$ in the positive ion MS/MS spectrum, which was

* Corresponding author. Tel.: +81 749 64 8101; fax: +81 749 64 8140.
E-mail address: s_ohta@nagahama-i-bio.ac.jp (S. Ohta).

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Table 1
NMR spectral data of **1** in D_2O–CD_3OD (9:1).[a]

No.	δ_C	δ_H multiplicity (J in Hz)	HMBC
2	157.6 s		
4	153.6 s		
5	104.5 s		
6	154.5 s		
8	144.9 d	8.12 s	C-4, C-5, C-1″
1′	42.6 t	4.52 dd (15.8, 7.0)	C-2, C-4, C-2′, C-3′
		4.56 dd (15.8, 7.0)	C-2, C-4, C-2′, C-3′
2′	118.5 d	5.12 br t (7.0)	C-4′, C-5′
3′	138.9 s		
4′	25.8 q	1.65 br s (3H)	C-2′, C-3′, C-5′
5′	18.4 q	1.75 br s (3H)	C-2′, C-3′, C-4′
1″	87.9 d	5.57 d (8.4)	C-5, C-8, C-2″, C-3″
2″	73.3 d	3.70 m	C-1″, C-3″
3″	76.5 d	3.68 m	C-2″, C-4″
4″	69.0 d	3.76 t (9.5)	C-3″, C-5″
5″	79.9 d	3.78 m	C-4″
6″	60.0 t	3.94 dd (12.5, 2.2)	C-4″, C-5″
		3.90 dd (12.5, 1.8)	C-4″, C-5″

[a] The ^1H and ^{13}C NMR were measured at 400 and 100 MHz, respectively. Chemical shifts were referenced to CD_3OD (δ_H 3.30 and δ_C 49.0).

established on the basis of the HMBC correlations (H_2-1′/C-2′ and C-3′; H-2′/C-4′ and C-5′; H_3-4′/C-2′, C-3′, and C-5′; H_3-5′/C-2′, C-3′, and C-4′). The HMBC correlations from H-1′ to C-2 and C-4 indicated the attachment of the isopentenyl unit at N-3 of the isoguanine unit. The interpretation of ^1H NMR and ^1H–^1H COSY spectra revealed the presence of a glucopyranosyl unit which was supported by the observation of a fragment ion peak at m/z 152 $[M-67-162]^+$ in the positive ion MS/MS spectrum. The HMBC correlations (Fig. 1) from H-1″ to C-5 and C-8 and from H-8 to C-1″ as well as the ^1H vicinal coupling constant of the anomeric proton indicated the attachment of the glucopyranosyl unit at N-7 of the isoguanine unit via an *N*-β-glycosidic linkage. The structure of **1** was confirmed by X-ray crystallographic analysis (Fig. 2).

Absolute configuration of glucose obtained after acid hydrolysis of **1** was determined as D by GC–MS analysis of the trimethylsilylated thiazolidine derivative (Hara et al., 1987). Consequently,

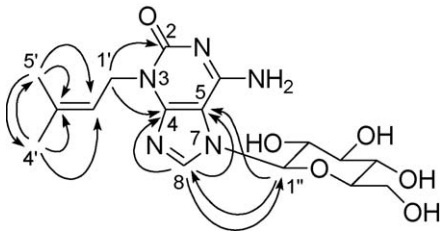

Fig. 1. Key HMBC correlations observed for **1**.

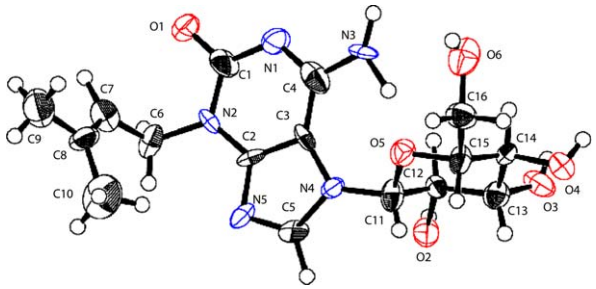

Fig. 2. Perspective view of the crystal structure of **1**.

the structure of locustoside A was elucidated as 7-β-D-glucopyranosyl-3-(3-methyl-2-butenyl)-isoguanine (**1**).

Most of naturally occurring prenylated purine alkaloids are N^6-prenylated adenine derivatives, e.g. N^6-isopentenyladenine (Robins et al., 1967), which are known as plant cytokinins. So far, only a few 3-prenylated purine alkaloids have been reported: triacanthine (Belikov et al., 1954; Leonard and Deyrup, 1962), saikachinoside A (Kajimoto et al., 2010), and dioicine (Fitch et al., 2009) have been isolated from the leaves of *Gleditsia triacanthos*, the seeds of *G. japonica*, and the leaves and seeds of the Kentucky coffeetree, *Gymnocladus dioicus*, respectively.

3. Experimental

3.1. General

The optical rotation was measured using a Horiba SEPA-300 polarimeter. UV and IR spectra were recorded on a JASCO V-630 and a Horiba FT720 spectrometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL AL400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C) at 25 °C. High-resolution ESITOFMS was measured on a Shimadzu LCMS-IT-TOF mass spectrometer. GC–MS were measured on a Shimadzu GCMS-QP2010 Plus mass spectrometer. Single-crystal X-ray analysis was carried out on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Mo Kα radiation (λ = 0.71075 Å). Column chromatography was carried out using ODS (Wacogel LP40C18). TLC was carried out on precoated Kieselgel 60 F_{254} (Merck, Darmstadt, Germany) and RP_{18} F_{254} plates (Merck).

3.2. Plant material

The seeds of *G. japonica* were collected in Shiga Prefecture, Japan in March 2009. Voucher specimens (No: SS090301) are kept at Nagahama Institute of Bio-Science and Technology.

3.3. Extraction, isolation and characterization

The seeds (166 g) of *G. japonica* were cut into small pieces and extracted with MeOH. The concentrated MeOH extract (10 g) was suspended in water and partitioned successively with hexane and EtOAc. The aqueous layer was lyophilized to yield syrup. The water-soluble fraction (8.4 g) was separated on an ODS column (50 g, 2.5 cm × 30 cm) employing MeOH in H_2O gradient mixtures [20:80, 40:60, 60:40, 80:20, 100:0 (100 mL of each solvent mixture)] yielding 10 fractions. Fraction 6 (38 mg, eluted with MeOH–H_2O, 60:40) was applied to an ODS column (4 g, 0.8 cm × 20 cm) with MeOH–H_2O [40:60, 60:40, 80:20 (10 mL of each solvent mixture)] to afford **1** (8.8 mg, 0.0053%).

3.4. Locustoside A (1)

Colorless needles; mp 209–212 °C; $[\alpha]_D^{25}$ +35.2° (*c* 0.44, H_2O); UV (MeOH) 246 (log ε 3.86), 290 nm (log ε 3.67); IR ν_{max} (KBr): 3600–3000, 1641, 1616 and 1583 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data see Table 1; HRESITOFMS m/z 382.1716 $[M+H]^+$ (calc. for $C_{16}H_{24}N_5O_6$, 382.1727).

3.5. X-ray crystallographic study of 1

Formula $C_{16}H_{23}N_5O_6$, M = 381.39, 0.40 mm × 0.20 mm × 0.10 mm, orthorhombic, space group $P2_12_12_1$ (#19), Z = 4, a = 8.298 (3) Å, b = 12.837 (4) Å, c = 18.160 (8) Å, $\alpha = \beta = \gamma = 90°$, V = 1934.4 (12) $Å^3$, $F(0\,0\,0)$ = 808, μ(Mo Kα) = 1.015 cm^{-1}, Dc = 1.309 g cm^{-3}, T = 100 K. 18 662 reflections measured, 2507

unique reflections, 715 observed reflections [$I > 2.0\sigma(I)$], $R = 0.0536$, $Rw = 0.0542$. CCDC 775672 contains the supplementary crystallographic data. The data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. Fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

3.6. Acid hydrolysis of 1

Treatment of **1** (1 mg) with 1 M HCl (100 μL) at 90 °C overnight gave a reaction mixture. The mixture, after drying, was dissolved in pyridine and L-cysteine methyl ester hydrochloride (1.6 mg) in pyridine (200 μL) was added. The mixture was heated at 60 °C for 1 h. The solution was then treated with 25% *N*,*O*-bis(trimethylsilyl)acetamide in acetonitrile solution (200 μL) at room temperature for 1 h. The supernatant was applied to GC–MS analysis [conditions: column, Rtx-5MS (Restek, USA), 30 m × 0.25 mm, 1 μm; carrier gas He; injection temperature 300 °C, column temperature 150 °C, 10 °C/min to 300 °C; t_R of derivatives, D-glucose 17.2 min, L-glucose 17.5 min]. D-Glucose was detected from **1**.

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