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# Saikachinoside A, a novel 3-prenylated isoguanine glucoside from seeds of *Gleditsia japonica*

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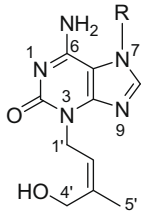
ABSTRACT

A new isoguanine glucoside designated saikachinoside A has been isolated from the seeds of *Gleditsia japonica*. The structure was determined as 7-β-D-glucopyranosyl-3-[(*Z*)-4-hydroxy-3-methyl-2-butenyl]isoguanine by interpretation of the spectroscopic data and was confirmed by X-ray crystallographic analysis. Saikachinoside A accelerated acetylcholinesterase activity.



The naturally occurring isopentenyl derivatives of purines presently known consist of triacanthine[1] and isoprenoid cytokinins[2,3] such as $N^6$-isopentenyladenine,[4] *trans*-zeatin,[5] *cis*-zeatin,[6,7] dihydrozeatin,[8] and their sugar conjugates.[9–11] In the course of our continuing search for biologically active compounds from plants,[12,13] we isolated a new 3-prenylated isoguanine[14] glucoside designated saikachinoside A (**1**) from the MeOH extract of seeds of Japanese honey locust, *Gleditsia japonica* Miquel (Leguminosae) which have been used as diuretics and expectorants in oriental traditional medicine.[15] This report describes the purification and the structure elucidation of **1**.




**1** R = β-D-Glucopyranosyl
**2** R = H

The pods of *G. japonica* were collected in Shiga Prefecture, Japan in March 2009. Voucher specimens are kept at Nagahama Institute of Bio-Science and Technology. The seeds (80 g, wet weight) 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 syrup. The water-soluble fraction (2.4 g) was separated repeatedly on an ODS column employing MeOH in $H_2O$ gradient mixtures to afford **1** (27 mg, 0.03% wet weight) as colorless plates, mp 205–208 °C, $[\alpha]_D^{25}$ +9.0 (*c* 1.1, $H_2O$).

Saikachinoside A (**1**) exhibited pseudomolecular ion peaks at *m/z* 398 and 396 corresponding to $[M+H]^+$ and $[M-H]^-$ in the (+)- and (−)-ESIMS, respectively. The molecular formula of **1** was established to be $C_{16}H_{23}N_5O_7$ on the basis of high-resolution ESITOFMS data (*m/z* 398.1677 $[M+H]^+$, Δ +0.1 mmu). The IR spectrum displayed absorption bands at 3600–2600 (OH, NH, and CH), 1635, 1626, and 1577 $cm^{-1}$ (C=O, C=N, and C=C). The UV ($H_2O$) absorption maxima at 246 (log ε 4.03) and 289 nm (log ε 4.00) suggested the presence of a 3,7-disubstituted isoguanine skeleton.[16,17] The presence of the isoguanine unit was supported by the $^1H$ and $^{13}C$ NMR (DMSO-$d_6$) chemical shifts [$\delta_H$ 8.06 (1H, s, H-8), $\delta_C$ 154.5 (C-2), 152.6 (C-4), 102.4 (C-5), 153.1 (C-6), 142.5 (C-8)]. Inspection of the $^1H$ and $^{13}C$ NMR spectra (Table 1) together with DEPT and HMQC spectral data revealed the presence of an olefinic methyl, three hetero atom bearing methylenes, five oxygenated methines, an olefinic methine, and a quaternary olefinic carbon in addition to the isoguanine unit. The interpretation of $^1H$ vicinal *J*-coupling constants and $^1H$–$^1H$ COSY spectrum revealed the presence of a monosaccharide unit which was supported by the observation of the fragment ion peak at *m/z* 234 $[M-163]^-$ in the negative ion MS/MS spectrum. The large vicinal coupling constants of H-1″ to H-5″ implied that these protons are all axial, indicating the monosaccharide unit to be β-glucopyranosyl. The HMBC (Fig. 1) from H-1″ to C-5 and C-8 and from H-8 to C-1″ indicated the attachment of the β-glucopyranosyl unit at N-7 of the isoguanine unit. The find-

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**Table 1**
NMR spectral data of **1** in DMSO-$d_6$ at 40 °C[a]

| No | $\delta_C$ | $\delta_H$ multiplicity ($J$ in Hz) | HMBC |
|---|---|---|---|
| 2 | 154.5 s | | |
| 4 | 152.6 s | | |
| 5 | 102.4 s | | |
| 6 | 153.1 s | | |
| 8 | 142.5 d | 8.06 s | C-4, C-5, C-1″ |
| 1′ | 39.3 t | 4.59 d (2H, 7.0) | C-2, C-4, C-2′, C-3′ |
| 2′ | 120.7 d | 5.26 br t (7.0) | C-4′, C-5′ |
| 3′ | 139.0 s | | |
| 4′ | 59.9 t | 4.11 d (2H, 5.5) | C-2′, C-3′, C-5′ |
| 5′ | 21.2 q | 1.68 br s (3H) | C-2′, C-3′, C-4′ |
| 1″ | 86.2 d | 5.40 d (8.4) | C-5, C-8, C-2″, C-3″ |
| 2″ | 72.3 d | 3.33 br dd (9.5, 8.4) | C-3″ |
| 3″ | 76.2 d | 3.35 br t (9.5) | C-2″ |
| 4″ | 68.0 d | 3.46 br t (9.5) | C-3″, C-6″ |
| 5″ | 79.2 d | 3.51 dt (9.5, 2.2t) | C-1″, C-3″, C-4″ |
| 6″ | 58.9 d | 3.67 br s (2H) | C-5″ |
| 6-$NH_2$ | | 7.08 br s (2H) | |
| 4′-OH | | 4.72 t (5.5) | C-3′, C-4′ |
| 2″-OH | | 5.44 br s | |
| 3″-OH | | 5.21 br s | |
| 4″-OH | | 5.15 br s | |
| 6″-OH | | 4.90 br s | C-5″ |

[a] The $^1H$ and $^{13}C$ NMR were measured at 400 and 100 MHz, respectively. Chemical shifts were referenced to solvent ($\delta_H$ 2.49 and $\delta_C$ 39.5).

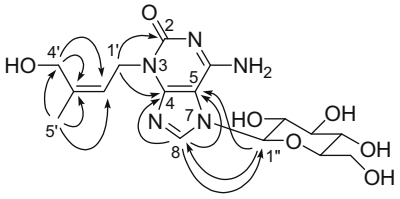


**Figure 1.** Key HMBC correlations observed for **1**.

ings were supported by an NOE experiment in which the enhancement of 5% on the anomeric proton (H-1″) was observed upon irradiation of H-8 (Fig. 2).

The presence of a 4-hydroxyisopentenyl unit was established on the basis of the HMBC ($H_2$-1′/C-2′ and C-3′; H-2′/C-4′ and C-5′; $H_2$-4′/C-2′, C-3′, and C-5′; $H_3$-5′/C-2′, C-3′, and C-4′). The HMBC from H-1′ to C-2 and C-4 indicated the attachment of the 4-hydroxyisopentenyl unit at N-3 of the isoguanine unit. The NMR chemical shifts of $H_3$-5′ ($\delta_H$ 1.68) and C-5′ ($\delta_C$ 21.2) suggested the geometry of $\Delta^{2'}$ to be *Z* [2-Me of (2*Z*,6*E*)-2,6-dimethyl-2,6-octadiene-1,8-diol: $\delta_H$ 1.79 and $\delta_C$ 21.1[18]] and not to be *E* [2-Me of (*E*)-4-phenyl-2-methyl-2-buten-1-ol: $\delta_H$ 1.80 and $\delta_C$ 13.8[19]]. The geometry was supported by difference NOE experiments. Enhancements of the oxygenated methylene protons ($H_2$-4′) and the olefinic proton (H-2′) upon irradiations of the allyl protons ($H_2$-1′) and the methyl protons ($H_3$-5′), respectively, were observed as shown in Figure 2. The structure of **1** was confirmed by X-ray crystallographic analysis (Fig. 3).[20]

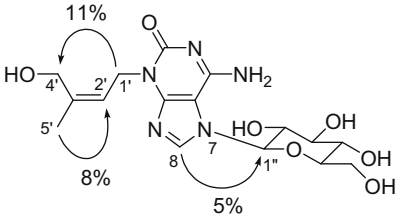


**Figure 2.** Key NOEs observed for **1**.

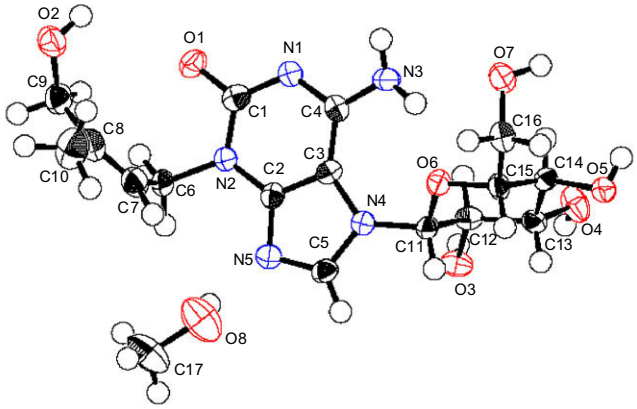


**Figure 3.** Perspective view of the crystal structure of **1**.

Hydrolysis of **1** with 1 M HCl afforded aglycone **2** and D-glucose.[21] Consequently, the structure of saikachinoside A was elucidated as 7-β-D-glucopyranosyl-3-[(*Z*)-4-hydroxy-3-methyl-2-butenyl]isoguanine (**1**).

Although several naturally occurring prenylated purines such as isoprenoid cytokinins have been reported, most of them are $N^6$-prenylated adenine derivatives.[2–8] There are very few, if any, compounds having a 3-prenylated purine skeleton.[1,22]

Saikachinoside A (**1**) was evaluated using electric eel acetylcholinesterase (AChE) following the colorimetric method of Ellman et al.[23] with some modifications.[24–26] The addition of $2.5 \times 10^{-4}$ M of **1** led to 1.3-fold increase in the AChE activity. It has been reported that several compounds, such as polyamines, activate AChE by allosteric mechanism.[27,28]

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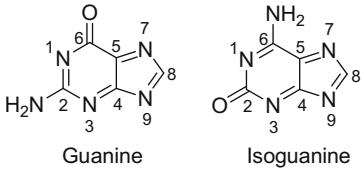
14. The structural formulae of guanine and isoguanine and their numbering schemes are defined as follows:

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20. Crystal data for **1**: $C_{16}H_{23}N_5O_7{\cdot}CH_3OH$, $M$ = 429.43, orthorhombic, space group $P2_12_12_1$ (#19), $Z$ = 4, $a$ = 8.358 (3), $b$ = 13.532 (4), $c$ = 17.820 (7) Å, $V$ = 2015.6 (12) Å$^3$, $F$(0 0 0) = 912, μ(Mo-Kα) = 1.130 cm$^{-1}$, $Dc$ = 1.415 g cm$^{-3}$, $T$ = 93 K. The reflection data were collected on a Rigaku RAXIS RAPID imaging plate area detector with graphite-monochromated Mo-Kα radiation (λ = 0.71075 Å). A total of 4609 independent reflections were collected of which 3192 were considered to be observed [$I > 2.0\sigma(I)$]. The structure was solved by direct methods and expanded using Fourier techniques.[29] The non-hydrogen atoms were refined anisotropically by full-matrix least-squares refinement.[30] Hydrogen atoms were refined isotropically. The structure was finally refined to $R$ = 0.0330 (w$R_2$ = 0.0699). Crystallographic data for the structure in this Letter has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 756695. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK; Fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk).
21. Treatment of **1** (6 mg) with 1 M HCl at 90 °C overnight gave a reaction mixture. The mixture, after neutralization, was subjected to a short ODS column chromatography to afford aglycone **2** (2 mg) {ESIMS $m/z$ 236 [M+H]$^+$} and a monosaccharide (2 mg). The monosaccharide was identified to be D-glucose on the basis of co-HPLC (Wakosil 5NH$_2$ column, 75%CH$_3$CN–H$_2$O, RI detector) with an authentic sample and the specific optical rotation {[α]$_D$ +45 ($c$ 0.1, H$_2$O)}.
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