Rare sulfated purine alkaloid glycosides from *Bruchidius dorsalis* pupal case

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ABSTRACT

Three new sulfated isoguanine alkaloid glycosides, locustoside A disulfate (**1**), saikachinoside B disulfate (**2**), and saikachinoside A trisulfate (**3**), have been isolated from the pupal case of the wild bruchid seed beetle *Bruchidius dorsalis* (Chrysomelidae, Bruchinae) infesting the seed of *Gleditsia japonica* Miquel (Fabaceae) along with the known compounds **9**–**11**. The structures of the new compounds were determined through spectroscopic data and X-ray crystallographic analysis. The isolated compounds were evaluated for their inhibitory activity against starfish embryogenesis.

Keywords: Bruchid seed beetle; *Bruchidius dorsalis*; Pupal case; *Gleditsia japonica*; Sulfated purine alkaloid; blastulation inhibitor.

1. Introduction

Intact seeds of the Japanese honey locust *Gleditsia japonica* Miquel (Fabaceae) reportedly contain rare N^3-prenylated purine alkaloid glycosides, namely, locustosides A (**4**) (Kajimoto et al., 2010b) and B (**5**) (Harauchi et al., 2017) and saikachinosides A (**6**) (Kajimoto et al., 2010a), B (**7**), and C (**8**) (Harauchi et al., 2017). Larvae of the wild bruchid seed beetle *Bruchidius dorsalis* (Chrysomelidae, Bruchinae) infest dry mature seeds of *G. japonica* and build pupal cases with their secretion/excretion products in the seeds (Shimada et al., 2001; Kurota and Shimada, 2001; Hirose et al., 2013). Our previous examination of the aqueous extract of the pupal cases led to the isolation of three rare sulfated purine alkaloid glycosides, saikachinoside A monosulfate (**9**), saikachinoside A disulfate (**10**), and locustoside B disulfate (**11**) among which, **9** and **10** exhibited weak inhibitory activity against acid phosphatase (Harauchi et al., 2018). Further examination of the extract of the pupal cases resulted in the isolation of three new compounds designated as locustoside A disulfate (**1**), saikachinoside B disulfate (**2**), and saikachinoside A trisulfate (**3**). This paper describes the purification, structural elucidation, and biological activity of **1**–**3**.

2. Results and discussion

The pupal cases were cut into small pieces and successively washed with hexane, EtOAc, and MeOH. The residue was extracted with H_2O, and the obtained H_2O-soluble material was repeatedly separated on an octadecylsilyl (ODS) column using gradient mixtures of MeOH and H_2O to afford **1**–**3** together with **9**–**11** (Fig. 1).

Compound **1** was obtained as colorless crystals (CH_3CN–H_2O). Negative-ion HR-ESI-FT-MS analysis showed a $[M–H]^–$ ion at *m/z* 540.0709 (calcd. for $C_{16}H_{22}N_5O_{12}S_2^–$, 540.0712), which established the molecular formula of **1** as $C_{16}H_{23}N_5O_{12}S_2$. The divalent ion at *m/z* 269.5322 ($[M–2H]^{2–}$; calcd. for $C_{16}H_{21}N_5O_{12}S_2^{2–}/2$, 269.5320) in the HR-ESI-FT-MS spectrum indicated the presence of two sulfate groups. The IR absorption bands at 3336, 1631, 1585,

1257, and 1236 cm^{-1} suggested the presence of OH, NH, C=O, C=N, C=C, and sulfate (Ohta et al., 1994; Uno et al., 1996) functionalities. The UV (H_2O) absorption maxima at 286 (log ε 3.73) and 243 nm (log ε 3.78) implied 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 data [δ_H 8.15 (1H, *s*, H-8); δ_C 157.5 (C-2), 154.3 (C-4) , 105.6 (C-5), 154.1 (C-6), 145.5 (C-8)] (Table 1). The ^{13}C NMR spectrum also revealed the presence of two additional sp^2 carbon atoms at δ_C 139.0 (C) and 118.8 (CH), seven heteroatom-substituted carbons at δ_C 85.6 (CH), 79.3 (CH), 78.6 (CH), 75.7 (CH), 73.9 (CH), 60.0 (CH_2), and 42.7 (CH_2), and two vinylic methyl groups at δ_C 25.7 (CH_3) and 18.4 (CH_3). The presence of a prenyl unit was established on the basis of the ^1H–^1H COSY correlation between δ_H 4.69 (2H, d, J = 7.0 Hz, H_2-1') and δ_H 5.22 (1H, br t, J = 7.0 Hz, H-2') and the HMBC correlations from δ_H 1.78 (3H, br s, H-4') to δ_C 139.0 (C-3'), 118.8 (C-2'), and 25.7 (C-5'), and from δ_H 1.70 (3H, br s, H-5') to δ_C 139.0 (C-3'), 118.8 (C-2'), and 18.4 (C-4'). The interpretation of ^1H–^1H COSY data in conjunction with the vicinal coupling constants led to the presence of a β-glucopyranosyl unit (C-1"–C-6"). HMBC correlations from H-1" to C-8 and C-5 of the isoguanine unit indicated the attachment of the β-glucopyranosyl unit to *N*-7 of the isoguanine unit (Fig. 2). This was confirmed by the NOESY correlation between H-8 and the anomeric proton (H-1") (Fig. 2). The ^1H NMR spectrum was similar to that reported for locustoside A (Kajimoto et al., 2010b), except that the signals at δ_H 4.42 (H-2") and 4.57 (H-4") in the spectrum of **1** were significantly shifted downfield from δ_H 3.70 (H-2") and 3.76 (H-4"), respectively, compared to that of locustoside A, indicating the attachment of the *O*-sulfate group to C-2" and C-4" of the β-glucopyranosyl unit. This was supported by the fragment ion peaks at *m/z* 218 $[M–323]^-$ and 321 $[M–220]^-$ in the negative-ion MS/MS spectrum (Fig. 3). The structure of **1** was confirmed by X-ray crystallographic analysis (Fig. 4). From the anomalous dispersion of the sulfur atoms, the absolute configuration of the 2",4"-disulfated glucosyl unit in **1** was confirmed to be D, with the Flack parameter (Flack, 1983; Parsons et al., 2013) being refined to –0.05 (16). In the solid state, the anion of **1** was linked to one other via sodium cations to form

polymeric chains along the *a* axis. The polymeric structures were stabilized by hydrophobic interactions between the prenyl groups, and π–π stacking interactions between the purine rings (Fig. 5). The interplanar spacing between the centroids of parallel six-membered rings in the purine skeletons was ~3.5 Å. Consequently, **1** is 7-[*β*-D-2,4-bis(sulfonyloxy)glucopyranosyl]-3-(3-methyl-2-butenyl)-isoguanine, to which the name locustoside A disulfate was given.

Compound **2** was obtained as a colorless oil; its molecular formula was established as $C_{21}H_{31}N_5O_{17}S_2$ by negative-ion HR-ESI-FT-MS analysis {*m/z* 688.1082 ($[M–H]^-$; calc. $C_{21}H_{30}N_5O_{17}S_2^-$, 688.1084)}. Moreover, the HR-ESI-FT-MS of **2** showed a divalent ion at *m/z* 343.5502 ($[M–2H]^{2-}$; calc. $C_{21}H_{29}N_5O_{17}S_2^{2-}$/2, 343.5506), indicating the presence of two sulfate groups. The ^{1}H and ^{13}C NMR spectra were similar to those of **1** (Table 1), except for presence of additional signals attributable to a pentosyl and an oxygenated methylene moiety, and the absence of one of the vinylic methyl signals of the N^3-prenyl group. By comparing the ^{13}C NMR chemical shifts for the pentosyl moiety with those previously reported in the literature (Harauchi et al., 2017, 2018; Kitagawa et al., 1993) and 2D NMR analysis (Fig. 2), the pentose was identified as β-apiofuranose. The NOESY correlation between the anomeric proton (H-1''') of the β-apiofuranosyl unit and the oxygenated methylene carbon (C-6'') of the glucopyranosyl unit (Fig. 2) as well as the downfield chemical shift of C-6'' of the glucopyranosyl unit indicated the attachment of the β-apiofuranosyl unit to C-6'' of the glucopyranose. Moreover, the ^{1}H and ^{13}C chemical shifts of C-2'' and C-4'' of the glucosyl unit of **2** were similar to those of **1**, indicating that the *O*-sulfate groups were attached to C-2'' and C-4'', similar to those in **1**. The presence of the di-*O*-sulfated disaccharide unit was supported by the fragment ion peaks at *m/z* 453, 373, and 234 in the negative-ion ESI-MS/MS spectrum (Fig. 3). The presence of a 4-hydroxyprenyl unit was established on the basis of the HMBC correlations from the oxygenated methylene proton (H-4') to C-2', C-3', and C-5' and from H-5' to C-2', C-3', and C-4'. The geometry of the double bond was assigned as *Z* on the basis of the NOE correlation between H-5' and H-2'. All other HMBC correlations also supported that **2** is the 2'',4''-di-*O*-sulfated analog of saikachinoside B (Harauchi et al., 2017) isolated from the seed of *G. japonica* (Fig. 2). The ECD

spectrum [Δε 224 (–3.9), 246 (+1.5), 286 nm (+1.3)] and the specific rotation value {$[\alpha]^{25}_{D}$ –7.1 (*c* 0.01, MeOH/H_2O, 7:3)} of **2** were almost the same as those of **11** {Δε 224 (–7.9), 247 (+1.9), 288 nm (+0.8); $[\alpha]^{25}_{D}$ –6.5 (*c* 0.4, MeOH/H_2O, 7:3)} described in the literature (Harauchi et al., 2018), indicating that the absolute configuration of **2** was identical to that of **11**. Hence, **2** is 7-[*β*-D-apiofuranosyl-(1–>6)-*β*-D-2,4-bis(sulfonyloxy)glucopyranosyl]-3-[(*Z*)-4-hydroxy-3-methyl-2-butenyl]isoguanine, to which the name saikachinoside B disulfate was given.

Compound **3** was obtained as a colorless oil; its molecular formula was determined to be $C_{16}H_{23}N_5O_{16}S_3$ by negative-ion HR-ESI-FT-MS analysis {*m/z* 636.0233 ($[M–H]^-$; calc. $C_{16}H_{22}N_5O_{16}S_3^-$, 636.0229)}. The trivalent ion at *m/z* 211.3364 ($[M–3H]^{3-}$; calc. $C_{16}H_{20}N_5O_{16}S_3^{3-}/3$, 211.3361)} as well as the divalent ion at *m/z* 317.5080 ($[M–2H]^{2-}$; calc. $C_{16}H_{21}N_5O_{16}S_3^{2-}/2$, 317.5078)} in the HR-ESI-FT-MS spectrum indicated the presence of three sulfate groups. The 1D and 2D NMR spectra (Table 1 and Fig. 2) were very similar to those of **10** described in the literature (Harauchi et al., 2018), except that the ^1H and ^{13}C signals of C-6" of the glucosyl unit in the spectrum of **3** were shifted downfield from δ_H 4.05/3.94 and δ_C 59.7 to δ_H 4.47/4.42 and δ_C 66.5, respectively, indicating the attachment of an additional *O*-sulfate group to C-6". This was supported by ESI-MS/MS data (Fig. 3). The geometry of the double bond was assigned as *Z* on the basis of the NOE correlation between the methyl protons (H-5') and the olefinic proton (H-2') (Fig. 2). The ECD spectrum [Δε 224 (–5.7), 244 (+1.7), 284 nm (+0.7)] and the specific rotation value {$[\alpha]^{25}_{D}$ +22.2 (*c* 1.0, H_2O)} of **3** were almost the same as those of **10** {Δε 219 (–4.5), 246 (+1.3), 284 nm (+0.5); $[\alpha]^{25}_{D}$ +28.2 (*c* 1.7, H_2O)} described in the literature (Harauchi et al., 2018), indicating that the absolute configuration of the 2",4"-di-*O*-sulfated glucosyl unit in **3** is D. Hence, **3** is 7-[*β*-D-2,4,6-tris(sulfonyloxy)glucopyranosyl]-3-[(*Z*)-4-hydroxy-3-methyl-2-butenyl]isoguanine, to which the name saikachinoside A trisulfate was given.

Thus, three new sulfated isoguanine alkaloid glycosides, namely, **1**, **2**, and **3** were isolated from the pupal case produced by the seed-eating larva of the bruchid beetle *B. dorsalis* inside the seed of *G. japonica*. Although some sulfated guanosine analogs, such as the kainate receptor inhibitor HF-7 (McCormick et al.,

1999), have been isolated from the venom of spiders (Taggi et al., 2004), sulfated nucleoside derivatives from natural sources other than spiders are rare. As **1**–**3** are not present in the intact seeds of *G. japonica*, they were presumably formed by regiospecific sulfation of the purine alkaloid glycosides **4**, **7**, and **6**, respectively, in the seed of *G. japonica* by the seed-eating larvae of *B. dorsalis*. Although further biological evaluation of the compounds is necessary, the ability of the larvae of *B. dorsalis* to sulfate the seed constituents might be related to the host-specificity of the bruchid beetle.

The biological activity of **1**–**3** was evaluated against starfish embryogenesis and compared to that of five related compounds **5**, **6**, and **9**–**11**. When fertilized *A. pectinifera* eggs were exposed to 200 μM of **1**, **2**, **5**, **6**, or **9**, they developed to the gastrula stage. In contrast, when fertilized *A. pectinifera* eggs were cultured from fertilization in the presence of **3** , **10**, or **11**, the development proceeded normally to the early blastula stage but the embryos ceased to develop further without hatching with an MIC of 75, 55, or 133 μM, respectively. It has been reported that adenosine inhibited blastulation during starfish embryonic development and caused significant suppression of the rate of protein, DNA, and RNA syntheses, whereas guanosine, uridine, cytidine, or thymidine did not affect blastulation (Tsuchimori et al. 1987, 1988). At the beginning of starfish blastulation, embryonic cells differentiate into epithelial cells to form a blastula. Although detailed molecular mechanisms of the developmental changes in the starfish embryos are still unknown, new inhibitors of blastulation, such as **3**, are considered to advance the studies on such molecular mechanisms.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a JASCO P-2200 polarimeter. Melting point was recorded on a Round Science RFS 10 melting point apparatus. The UV and IR spectra were recorded on JASCO FT/IR-6300 and JASCO V-630 Bio spectrophotometer, respectively. The ECD spectra was measured on a JASCO J-

725 spectropolarimeter. NMR spectra were acquired using a JEOL AL400 NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). ^{1}H and ^{13}C NMR chemical shifts were referenced to residual solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD. MS data were obtained on a Thermo Fisher Scientific LTQ Orbitrap XL mass spectrometer at the Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University. Column chromatography (CC) was performed using Wakogel 50C18 (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Thin layer chromatography (TLC) was performed using pre-coated silica gel RP-18 F_{254}s plates (Merck).

3.2. Plant material

The pupal cases built by *Bruchidius dorsalis* (Chrysomelidae, Bruchinae) inside the seeds of *Gleditsia japonica* Miquel (Fabaceae) were collected as described in the literature (Harauchi et al., 2018). Voucher specimens of *B. dorsalis* (registry number HUM-Ins-0004263) and the infested seed of *G. japonica* (registry number HUM-PL-00003) have been deposited at the Hiroshima University Museum, Japan.

3.3. Extraction and isolation

H_2O-soluble material (13 g) of the pupal cases (127 g) was obtained as described in the literature (Harauchi et al., 2018). A portion (1.5 g) of the H_2O-soluble material (13 g) was subjected to medium pressure CC on ODS with gradient mixtures of MeOH–H_2O (0:100 to 100:0). The first fraction (100% H_2O, 83 mg) was purified by CC on ODS using H_2O as an eluent to afford **1** (3 mg), **2** (6 mg), and **3** (30 mg).

*3.3.1. Locustoside A disulfate (**1**)*

Colorless crystals; m.p. 257–259 ˚C; $[\alpha]_D^{25}$ +4.4˚ (*c* 0.16, H_2O); UV (H_2O) λ_{max} (log ε) 286 (3.73) and 243 nm (3.78); IR (film) ν_{max} 3336, 1631, 1585, 1257, and 1236 cm^{-1}; ECD (H_2O) $\Delta\varepsilon_{226}$ –2.5, $\Delta\varepsilon_{246}$ +0.7, and $\Delta\varepsilon_{288}$ +0.3; ^{1}H NMR, see

Table 1; ^{13}C NMR, see Table 1; (–)HR-ESI-FT-MS *m/z* 540.0709 $[M–H]^-$ (calcd for $C_{16}H_{22}N_5O_{12}S_2^-$, 540.0712) and 269.5322 $[M–2H]^{2-}$ (calcd for $C_{16}H_{21}N_5O_{12}S_2^{2-}$/2, 269.5320).

*3.3.2. Saikachinoside B disulfate (**2**)*

Colorless oil; $[\alpha]_D^{25}$ –7.1˚ (*c* 0.01, MeOH/H_2O, 7:3); UV (H_2O) λ_{max} (log ε) 288 (3.76) and 245 nm (3.83); IR (film) ν_{max} 3380, 1668, 1640, 1588, 1261, and 1233 cm^{-1}; ECD (H_2O) $\Delta\varepsilon_{224}$ –3.9, $\Delta\varepsilon_{246}$ +1.5, and $\Delta\varepsilon_{286}$ +1.3; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; (–)HR-ESI-FT-MS *m/z* 688.1082 $[M–H]^-$ (calcd for $C_{21}H_{30}N_5O_{17}S_2^-$, 688.1084) and 343.5502 $[M–2H]^{2-}$ (calcd for $C_{21}H_{29}N_5O_{17}S_2^{2-}$/2, 343.5506).

*3.3.3. Saikachinoside A trisulfate (**3**)*

Colorless oil; $[\alpha]_D^{25}$ +22.2˚ (*c* 1.0, H_2O); UV (H_2O) λ_{max} (log ε) 289 (3.79) and 246 nm (3.86); IR (film) ν_{max} 3443, 3353, 1633, 1586, 1255, and 1232 cm^{-1}; ECD (H_2O) $\Delta\varepsilon_{224}$ –5.7, $\Delta\varepsilon_{244}$ +1.7, and $\Delta\varepsilon_{284}$ +0.7; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; (–)HR-ESI-FT-MS *m/z* 636.0233 $[M–H]^-$ (calcd for $C_{16}H_{22}N_5O_{16}S_3^-$, 636.0229), 317.5080 $[M–2H]^{2-}$ (calcd for $C_{16}H_{21}N_5O_{16}S_3^{2-}$/2, 317.5078), and 211.3364 $[M–3H]^{3-}$ (calcd for $C_{16}H_{20}N_5O_{16}S_3^{3-}$/3, 211.3361).

*3.4. X-ray crystallographic analysis of locustoside A disulfate (**1**)*

Data collection was performed with a Bruker SMART-APEX II ULTRA CCD area detector with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods using SHELXS-97 (Sheldrick, 2008). Refinements were performed with SHELXL-2014 (Sheldrick, 2014) using full-matrix least squares on F^2. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in idealized positions and refined as riding atoms isotropically. CCDC-1949825 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Crystal data for locustoside A disulfate (**1**): monoclinic, crystal size, ~~0.40 x 0.01 x 0.01~~ 0.20 x 0.10 x 0.02 mm^3, Space group *C* 1 2 1, Z = 8, crystal cell parameters *a* = 56.07 (4) Å, *b* = 5.443 (4) Å, *c* = 16.997 (11) Å, β = 90.774 (7)˚, V = 5187 (6) $Å^3$, F(000) = 2656, Dc = 1.653 Mg/m^3, T = 173 K, 12608 reflections measured, 7057 independent reflections [$R_{(int)}$ = 0.1239], final *R* indices [$I>2.0\sigma(I)$], R_1 = 0.1532, wR_2 = 0.3057; final *R* indices (all data), R_1 ~~value~~ = 0.3027, wR_2 = 0.3517, Flack parameter (Parsons et al., 2013): –0.05 (16).

3.5. Starfish gamete assay

The starfish embryogenesis inhibitory activity was evaluated by a modification of the method reported in the literature (Ohta et al., 2013). Adult starfish (*A. pectinifera*) specimens were collected from the coastal waters of Japan during breeding season and kept in seawater at 15 ˚C in laboratory aquaria. Experiments were performed at 20 ˚C using filtered seawater diluted to 90% (v/v) with distilled water. Oocytes and sperm were removed from ovarian and testicular fragments, respectively. Oocyte maturation was induced by treatment with 1 ~~mM~~ μM 1-methyladenine (Sigma, St. Louis, MO, USA) (Kanatani 1973). Maturing oocytes were fertilized by the addition of the diluted sperm suspension, at 40 min after the start of the 1-methyladenine treatment. Fertilized eggs were washed three times with seawater. The MeOH solution of the sample to be tested was added to the suspensions of embryos to achieve a final concentration of 1% MeOH in seawater. The MeOH concentration had no effect on embryonic development. The embryos were periodically observed for any cytological changes. Adenosine (Tsuchimori et al., 1988) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as a positive control, and H_2O was used as a negative control. All experiments were performed in duplicate.

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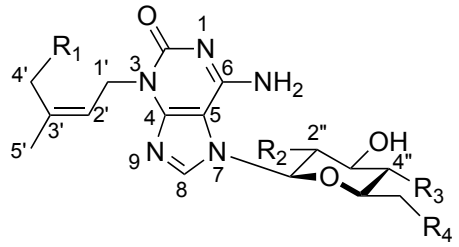
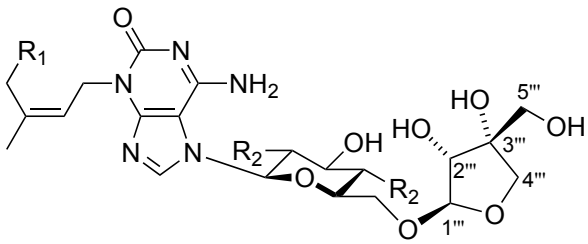
Table 1
NMR spectroscopic data for **1**–**3**.

No.	**1**[a]		**2**[a]		**3**[a]	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	157.5		154.6		157.3	
4	154.3		154.1		153.9	
5	105.6		104.7		105.5	
6	154.1		152.2		154.5	
8	145.5	8.15 (s)	147.0	8.30 (s)	145.5	8.16 (s)
1'	42.7	4.69 (d, 7.0)	42.0	4.78 (d, 7.0)	41.8	4.72 (br s)
2'	118.8	5.22 (br t, 7.0)	121.6	5.41 (br t, 7.0)	122.4	5.36 (br s)
3’	139.0		140.7		139.9	
4’	18.4	1.78 (br s)	61.2	4.28 (br s)	61.2	4.26 (br s)
5'	25.7	1.70 (br s)	21.4	1.77 (br s)	21.4	1.75 (br s)
1"	85.6	5.84 (d, 9.2)	85.7	5.91 (d, 9.2)	85.5	5.87 (d, 8.9)
2"	79.3	4.42 (dd, 9.5, 9.2)	80.3	4.35 (t, 9.2)	79.0	4.40 (t, 8.9)
3"	73.9	4.06 (t, 9.5)	73.9	4.07 (dd, 9.5, 9.2)	74.0	4.08 (dd, 9.2, 8.9)
4"	75.7	4.57 (t, 9.5)	75.7	4.70 (t, 9.5)	75.8	4.58 (t, 9.2)
5"	78.6	3.97 (br d, 9.5)	77.5	4.13 (br d, 9.5)	76.4	4.23 (br d, 9.2)
6"	60.0	4.02 (br d, 12.5)	66.1	4.07 (br d, 11.0)	66.5	4.47 (br d, 11.0)
		3.93 (br d, 12.5)		3.97 (br d, 11.0)		4.42 (br d, 11.0)
1'"			110.1	5.12 (d, 3.1)		
2'"			77.3	4.07 (d, 3.1)		
3'"			79.0			
4'"			74.4	4.01 (d, 10.4)		
				3.85 (d, 10.4)		
5'"			63.8	3.65 (br s)		

[a] Chemical shifts were measured in CD_3OD–D_2O (1:9) at 400 MHz for ^1H, 100 MHz for ^{13}C.



1 : R_1 = H, R_2 = R_3 = OSO_3H, R_4 = OH
3 : R_1 = OH, R_2 = R_3 = R_4 = OSO_3H
4 : R_1 = H, R_2 = R_3 = R_4 = OH
6 : R_1 = R_2 = R_3 = R_4 = OH
8 : R_1 = O-β-D-Glc, R_2 = R_3 = R_4 = OH
9 : R_1 = R_3 = R_4 = OH, R_2 = OSO_3H
10 : R_1 = R_4 = OH, R_2 = R_3 = OSO_3H



2 : R_1 = OH, R_2 = OSO_3H
5: R_1 = H, R_2 = OH
7: R_1 = R_2 = OH
11 : R_1 = H, R_2 = OSO_3H

Fig. 1. Structures of compounds **1**–**11**.

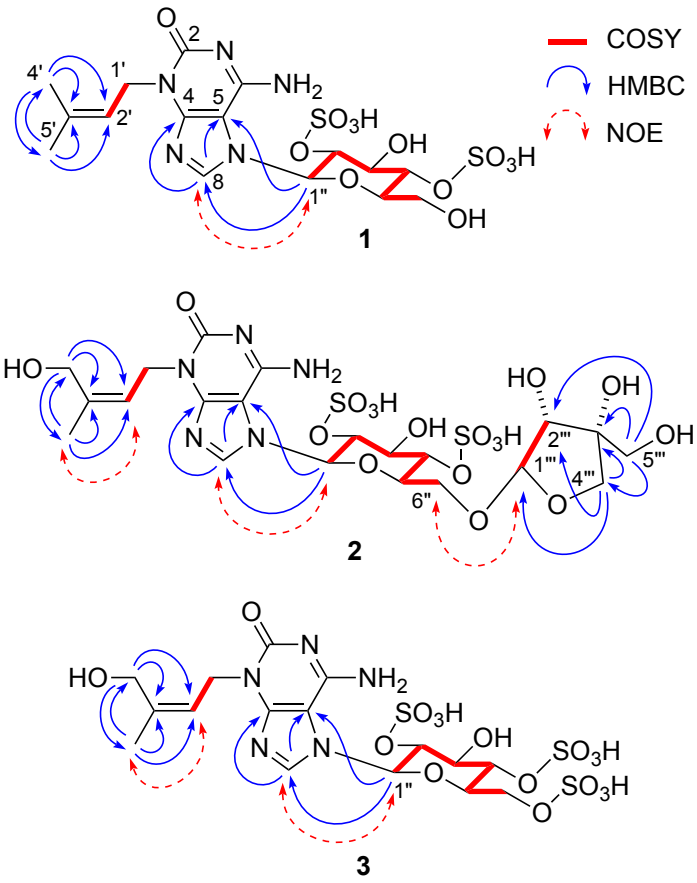


Fig. 2. COSY, HMBC, and NOE key data for **1**–**3**.

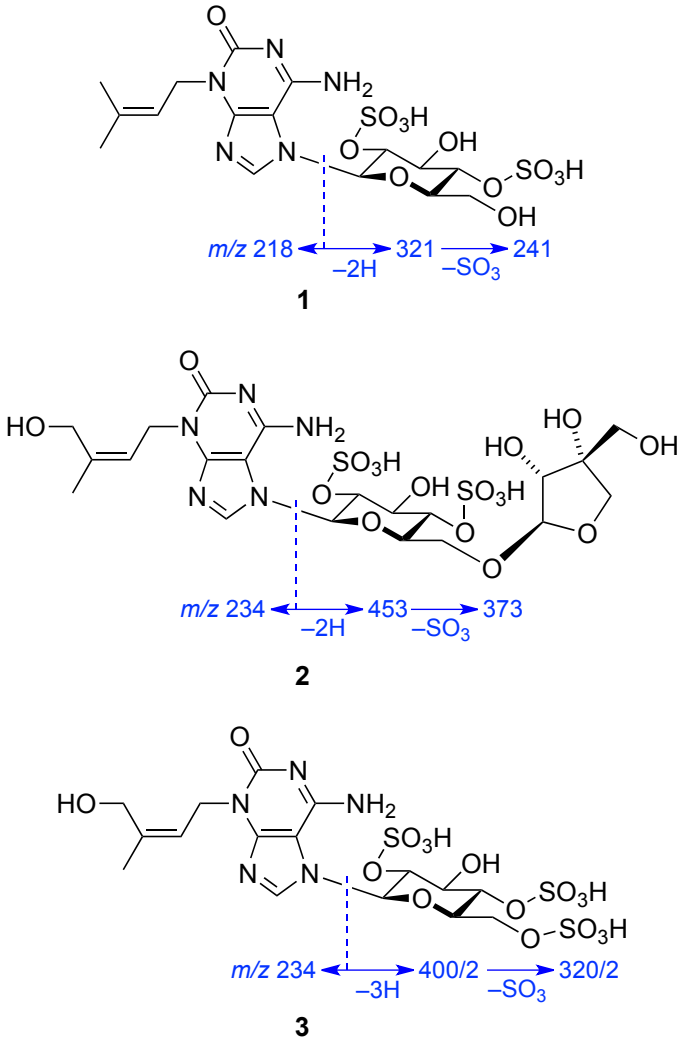


Fig. 3. Negative-ion ESI-MS/MS analysis of **1**–**3**.

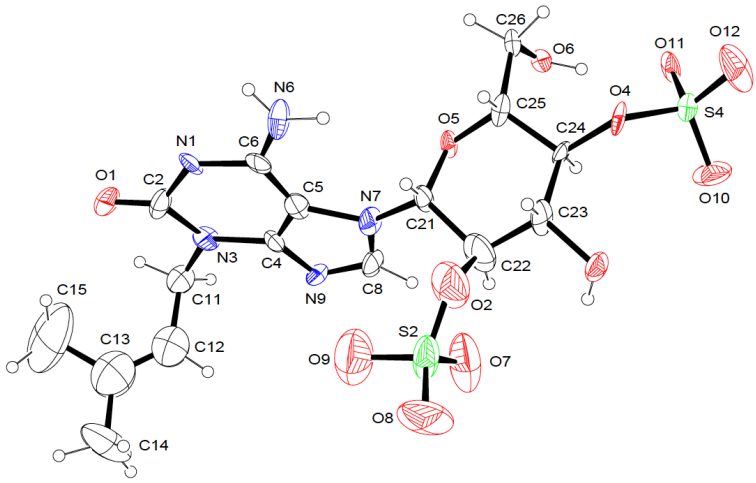


Fig. 4. ORTEP diagram of **1**. Solvent molecules and sodium ions are omitted for clarity.

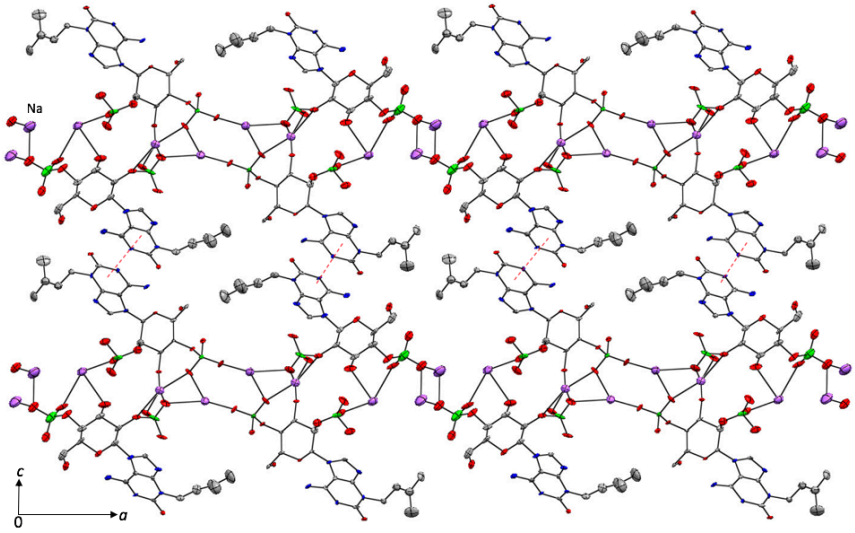


Fig. 5. A diagram, viewed down *b* axis, showing the π-π stacking interactions in **1**, marked as red dashed lines. Solvent molecules and hydrogen atoms are omitted for clarity.