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Deinococcucins A−D, Aminoglycolipids from *Deinococcus* sp., a Gut Bacterium of the Carpenter Ant *Camponotus japonicus*

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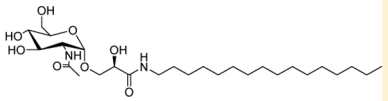
Supporting Information

ABSTRACT: Four new aminoglycolipids, deinococcucins A−D (**1**−**4**), were discovered from a *Deinococcus* sp. strain isolated from the gut of queen carpenter ants, *Camponotus japonicus*. The structures of deinococcucins A−D were elucidated as a combination of *N*-acetyl glucosamine, 2,3-dihydroxypropanoic acid, and an alkyl amine with a C_{16} or C_{17} hydrocarbon chain primarily based on 1D and 2D NMR and mass spectroscopic data. The exact location of the olefinic double bond in deinococcucins C and D (**3** and **4**) was assigned based on the liquid chromatography−mass spectroscopy data obtained after olefin metathesis. The absolute configurations of the *N*-acetyl glucosamine and 2,3-dihydroxy moieties were determined through gas chromatography−mass spectroscopy analysis of authentic samples and phenylglycine methyl ester-derivatized products, respectively. Deinococcucins A and C displayed significant induction of quinone reductase in murine Hepa-1c1c7 cells.

Camponotus japonicus



Deinococcucin A

Microbes in symbiotic insect ecosystems have recently been recognized as a tremendous reservoir of bioactive small molecules for drug discovery.[1−3] However, only a handful of insect systems have been investigated for microbial compounds, even though the class Insecta is estimated to contain approximately 5 million species in 28 orders.[4] Representative chemical studies on the bioactive small molecules produced by insect-associated microbes have involved the southern pine beetle *Dendroctonus frontalis*[5−7] and the dung beetle *Copris tripartitus*,[8−11] in the order Coleoptera, the fungus-growing termite *Macrotermes natalensis*[12−14] in the order Blattodea, the mantis *Tenodera aridifolia*[15] in the order Mantodea, the grasshopper *Oxya chinensis* in the order Orthoptera,[16,17] and the fungus-growing ants (*Apterostigma dentigerum* and *Trachymyrmex cornetzi*)[18,19] and the wasps (*Sceliphron caementarium*[20,21] and *Philanthus triangulum*[22,23]) in the order Hymenoptera.

Hymenoptera, which harbors more than 125 000 described species and 220 000 estimated species, is one of the four most speciose insects.[24] This order distinctively encompasses most of the eusocial insects. Ants are common and representative eusocial insects belonging to the family Formicidae in the order Hymenoptera.[25] Their successful adaptation in diverse environments has been explained by their organized social life cycles. As highlighted in the pioneering chemical studies of symbiotic bacteria in fungus-growing ants,[18,19] bacterial associations with ants can be a general phenomenon. Therefore, the chemical investigation of bacterial secondary metabolites in ant ecosystems may be an efficient strategy to discover new bioactive small molecules. This study focused on the ecosystem of the carpenter ant *Camponotus japonicus*, which is a dominant ant species in East Asia.[26] We collected carpenter ants in Seoul Grand Park, Republic of Korea, and isolated bacterial strains from the ant specimens. Chemical analysis of the bacterial metabolites by liquid chromatography−mass spectroscopy (LC/MS) revealed that strain SJN1, which was most closely related to *Deinococcus xinjiangensis* and isolated from the gut of queen specimens, produced a series of previously unreported compounds that were invisible under UV detection but distinctly detected in mass spectra ($[M + H]^+$ ions at m/z 531−547). Further scaling up the culture of SJN1 and chromatographic isolation of these compounds by HPLC with refractive index (RI) detection resulted in the discovery of four new aminoglycolipids, deinococcucins A−D (**1**−**4**). Here, we report the structural determination, including the absolute configurations, and the biological activities of deinococcucins A−D (**1**−**4**), rare metabolites from the genus *Deinococcus*.

RESULTS AND DISCUSSION

Deinococcucin A (**1**) was isolated as an amorphous, colorless oil and contained an $[M + H]^+$ ion at m/z 533.3756 in its high-resolution electrospray ionization mass spectroscopy (HRE-

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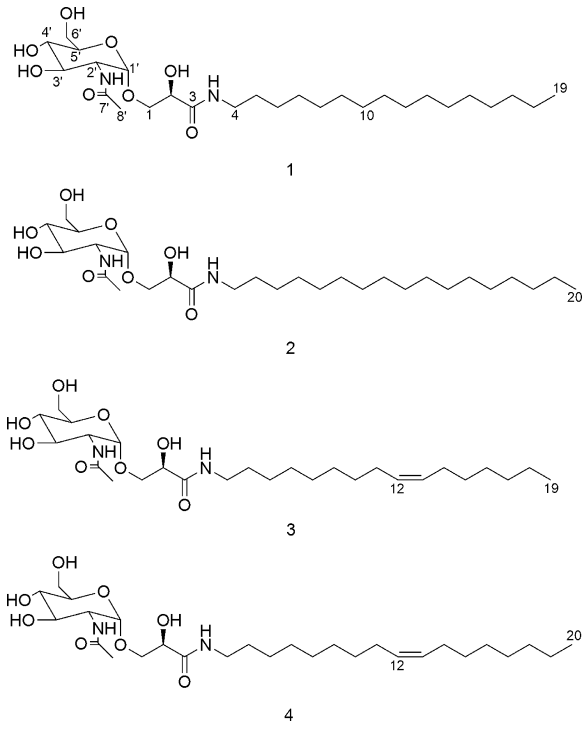

SIMS) data. The exact mass was consistent with the molecular formula $C_{27}H_{52}N_2O_8$, which provided three unsaturation equivalents. The infrared absorption peaks at 3360 and 1640 cm^{-1} indicated the presence of hydroxy and amide functional groups, respectively. The ^{13}C NMR data revealed the presence of 27 carbon signals, which were classified from the HSQC spectrum as two sp^3 methyl (δ_C 22.8 and 13.9), 17 sp^3 methylene (δ_C 69.6−29.1), and six sp^3 methine (δ_C 97.1, 72.6, 70.8, 70.8, 70.4, and 53.4) carbons and two carbonyl carbons (δ_C 171.1 and 169.3).

The $^1J_{CH}$ direct connectivities between protons and carbons were established by analyzing the HSQC spectrum and the tabulated ^1H and ^{13}C NMR spectral data for **1**, which are listed in Table 1. The planar structure of deinococcucin A (**1**) was elucidated by analyzing its COSY and HMBC data (Figure 1), revealing a hexose structure. The $^1H-^1H$ homonuclear couplings demonstrated the formation of a spin system from H-1′ (δ_H 4.62) to H_2-6′ (δ_H 3.55 and 3.47). H-1′/C-5′ and H-5′/C-1′ HMBC correlations established the closure of the hexose ring. The exchangeable protons of 2′-NH (δ_H 7.56), 3′-OH (δ_H 4.78), 4′-OH (δ_H 5.00), and 6′-OH (δ_H 4.45) were assigned in this hexose spin system based on the $^1H-^1H$ COSY correlations. In addition, the HMBC correlations from 2′-NH and H_3-8′ (δ_H 1.85) to the amide carbon C-7′ (δ_C 169.3) established an *N*-acetyl group at the C-2′ position. Another spin system was identified from the H-1/H-2 and H-2/2-OH COSY correlations. The $^2J_{CH}$ coupling from H-2 to the carbonyl carbon C-3 constructed a 2,3-dihydroxypropanoic acid moiety. The last partial structure was identified as an alkyl amine hydrophobic chain starting from the NH group. The amide proton 3-NH showed a COSY correlation to H_2-4. The lipophilic chain composed of highly overlapped methylene protons and a terminal methyl group (δ_H 0.85; δ_C 13.9) was deduced based on the molecular formula, the overlapped COSY correlations among the methylene protons, and the clear COSY correlation from a methylene proton (H_2-18) to the methyl group (H_3-19).

The three partial structures, a hexose ring, 2,3-dihydroxypropanoic acid, and an alkyl amine hydrocarbon chain, were assembled based on the HMBC correlations. The hexose ring was connected to 2,3-dihydroxypropanoic acid through an ether based on the H-1′/C-1 and H-1/C′-1 HMBC correlations. The HMBC correlations from H-2 and 3-NH to the carbonyl carbon C-3 at δ_C 171.1 secured the connectivity from 2,3-dihydroxypropanoic acid to the hydrocarbon chain unusually starting at an NH group and not at a carbonyl carbon. Therefore, the planar structure of deinococcucin A (**1**) was elucidated as a new aminoglycolipid (Figure 1).

Deinococcucin B (**2**) was purified as a colorless oil with a molecular formula of $C_{28}H_{54}N_2O_8$ determined from its HRESIMS data. The 1D and 2D NMR spectroscopic data of **2** (Table 1) displayed almost identical features to those of **1**. Careful analysis of the NMR data disclosed the presence of one additional sp^3 methylene carbon in the lipid chain of **2** compared to **1**, thus determining the structure of **2** as an analogue of **1** with a C_{17} alkyl chain.

Deinococcucin C (**3**) was obtained as a colorless oil, and its molecular formula was deduced to be $C_{27}H_{50}N_2O_8$ based on its HRESIMS data. Careful comparison of the NMR spectroscopic data (Table 2) revealed that the ^1H and ^{13}C NMR spectra of **3** in DMSO-d_6 were very similar to those of **1** but that deinococcucin C (**3**) bears two overlapped sp^2 methine carbons (δ_C 129.0), indicating that **3** possesses a double bond in the hydrocarbon chain. Further analysis of its 2D NMR spectra, including COSY, HSQC, and HMBC data, indicated that the structure of deinococcucin C (**3**) was analogous to that of **1** with an olefinic double bond in its chain. However, the overlapped ^1H and ^{13}C NMR resonances of the hydrocarbon chain hampered the determination of the exact position and the geometry of the double bond, which required further analysis.

For the exact assignment of the double-bond position, a recently developed method utilizing olefin cross-metathesis and subsequent LC/MS analysis was applied.[27] Cross-metathesis of **3** with methyl acrylate using the second-generation Hoveyda−Grubbs catalyst yielded a UV-detectable product (Figure 2). The double-bond positions could be simply deduced by comparing the mass changes between deinococcucin C ($[M + H]^+$ at m/z 531) and the cross-metathesis product in the LC/MS data (Figure 2). The molecular mass of the deinococcucin C-derived cross-metathesis product was 504 ($[M + H]^+$ at m/z 505), indicating a 26 dalton decrease from the mass of deinococcucin C. This result can be easily explained by the removal of a C_6H_{13} group (85 Da) and the addition of a CO_2CH_3 group (59 Da) in the metathesis partner, methyl acrylate, during cross-metathesis, thus placing the double-bond position at the seventh carbon from the chain terminus. Therefore, the double-bond position was unequivocally established between C-12 and C-13.

Regarding the geometry of the double bond, the $^1H-^1H$ coupling constant between H-12 and H-13 could not be measured because these two protons overlap at δ_H 5.33. Instead, the ^{13}C chemical shifts were used to establish the configuration of the olefinic bond. Reportedly, the aliphatic carbons flanking a *Z*-olefin in a long hydrocarbon chain resonate at δ_C 28−30, while those next to an *E*-double bond appear at δ_C 42−43, clearly distinguishing the *E*/*Z* geometries.[28,29] The chemical shifts of C-11 and C-14 were detected at δ_C 28.5, thus assigning the 12*Z* configuration.

Deinococcucin D (**4**) was isolated as a colorless oil. The molecular formula was assigned as $C_{28}H_{52}N_2O_8$ based on its

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Table 1. ^{1}H and ^{13}C NMR Data for Deinococcucins A (1) and B (2) in DMSO-d_6[a]

	deinococcucin A (1)		deinococcucin B (2)	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	69.6, CH_2	3.63, m	69.6, CH_2	3.63, m
		3.45, m		3.49, m
2	70.8, CH	3.97, dd	70.8, CH	4.00, dd
		(5.5, 3.0)		(5.5, 3.0)
3	171.1, C		171.1, C	
4	38.2, CH_2	3.02, m	38.5, CH_2	3.05, m
5	29.1, CH_2	1.35, m	29.4, CH_2	1.40, m
6	26.1, CH_2	1.22, m	26.4, CH_2	1.23, m
7−16	28.9−28.4, $10CH_2$	1.26−1.15, m	28.9−28.3, $10CH_2$	1.31−1.19, m
17	31.0, CH_2	1.37, m	28.3, CH_2	1.13, m
18	22.4, CH_2	1.24, m	31.2, CH_2	1.41, m
19	13.9, CH_3	0.85, t (6.5)	22.0, CH_2	1.20, m
20			14.0, CH_3	0.85, t (5.5)
2-OH		5.52, br s		5.50, br s
3-NH		7.76, t (5.5)		7.76, t (5.5)
1′	97.1, CH	4.62, d (3.5)	97.0, CH	4.61, d (3.5)
2′	53.4, CH	3.67, dd	53.4, CH	3.67, dd
		(8.5, 3.5)		(8.5, 3.5)
3′	70.8, CH	3.44, dd	70.7, CH	3.41, dd
		(10.0, 8.5)		(10.0, 8.5)
4′	70.4, CH	3.12, ddd	70.4, CH	3.16, ddd
		(10.0, 9.0, 2.5)		(10.0, 9.0, 2.5)
5′	72.6, CH	3.35, ddd	72.7, CH	3.37, ddd
		(9.0, 5.0, 2.5)		(9.0, 5.0, 2.5)
6′	60.6, CH_2	3.55, m	60.5, CH_2	3.57, m
		3.47, m		3.46, m
7′	169.3, C		169.2, C	
8′	22.8, CH_3	1.85, s	22.6, CH_3	1.85, s
2′-NH		7.56, d (8.5)		7.60, d (8.5)
3′-OH		4.78, br s		4.78, br s
4′-OH		5.00, br s		5.00, br s
6′-OH		4.45, dd		4.45, dd
		(12.0, 3.5)		(12.0, 3.5)

[a] ^{1}H and ^{13}C data were recorded at 600 and 125 MHz, respectively.

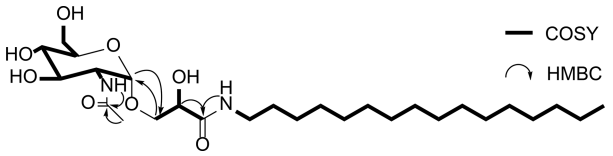

Figure 1. Key $^{1}H-^{1}H$ COSY and HMBC correlations of **1**.

HRESIMS data. Similar to the relationship between deinococcucins A (**1**) and C (**3**), deinococcucin D (**4**) has two more sp^2 methine (δ_C 129.6) and two less sp^3 methylene carbons compared to **2**. Detailed analysis of the 2D NMR spectra (Table 2) indicated that deinococcucin D also possesses an olefinic double bond in the lipid chain similar to **3**. The location and geometry of the double bond in **4** were determined as 12*Z* by cross-metathesis and ^{13}C chemical shift analysis, as shown for **3**.

The relative configuration of the hexose ring was established by analyzing the $^{1}H-^{13}C$ and $^{1}H-^{1}H$ coupling constants and the ROESY NMR through-space correlations. The magnitude of $^{1}J_{CH}$ (170 Hz) between C-1′ and H-1′ was clearly indicative of an α-configuration,[30] placing the anomeric proton (H-1′) in an equatorial position. The large $^{1}H-^{1}H$ vicinal coupling constant (8.5 Hz) between H-2′ and H-3′ established their *anti*-relationship and, therefore, axial positions. In addition, H-4′ was assigned an axial position based on $^{3}J_{HH}$ (10.0 Hz) between H′-3 and H′-4. Lastly, the $^{1}H-^{1}H$ coupling constant (9.0 Hz) of the H-5′ doublet determined its axial position, and the H-2′/H-4′ ROESY correlation confirmed their relative configurations. Therefore, the hexose moiety was determined to be *N*-acetyl glucosamine (Figure 3).

To determine the absolute configuration of the glucosamine moiety in deinococcucin A (**1**), the compound was subjected to acid hydrolysis, yielding glucosamine, which was derivatized using hexamethyldisilazane (HMDS) and TMS-Cl and analyzed by gas chromatography−mass spectroscopy (GC/MS). The derivative of glucosamine from *N*-acetyl glucosamine in **1** exhibited a retention time that was consistent with that of the authentic D-glucosamine derivative.[31]

To determine the absolute configuration of deinococcucin A (**1**), the phenylglycine methyl ester (PGME) method was applied.[32] Prior to this procedure, acid hydrolysis of **1** mainly yielded a 2,3-dihydroxypropanoic acid (**5**). The carboxylic acid group at C-1 in **5** was then derivatized with *S*- or *R*-PGME to yield the *S*- or *R*-PGME amide (**5a** and **5b**), respectively. The ^{1}H chemical shifts of the relevant protons were assigned by analyzing the ^{1}H NMR spectra of **5a** and **5b**. Calculating the

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Table 2. ^{1}H and ^{13}C NMR Data for Deinococcucins C (3) and D (4) in DMSO-d_6[a]

	deinococcucin C (3)		deinococcucin D (4)	
position	δ_C, type	δ_H, mult (J in Hz)	δ_C, type	δ_H, mult (J in Hz)
1	69.6, CH_2	3.63, m	69.6, CH_2	3.62, m
		3.49, m		3.47, m
2	70.8, CH	4.00, dd	70.6, CH	4.00, dd
		(5.5, 3.0)		(5.5, 3.0)
3	171.1, C		171.0, C	
4	38.2, CH_2	3.06, m	38.2, CH_2	3.05, m
5	29.1, CH_2	1.39, m	29.1, CH_2	1.40, m
6	26.1, CH_2	1.22, m	26.1, CH_2	1.22, m
7−10	28.9−28.4, 4CH_2	1.33−1.26, m	28.9−28.4, 4CH_2	1.35−1.25, m
11	28.5, CH_2	1.97, m	28.3, CH_2	1.95, m
12−13	129.0, 2CH	5.33, m	129.6, 2CH	5.32, m
14	28.5, CH_2	1.97, m	28.3, CH_2	1.95, m
15−17	31.0−29.9, 3CH_2	1.33−1.26, m	29.9−29.4, 3CH_2	1.35−1.25, m
18	22.4, CH_2	1.26, m	31.2, CH_2	1.28, m
19	13.9, CH_3	0.85, t (6.5)	22.0, CH_2	1.26, m
20			14.0, CH_3	0.84, t (6.5)
2-OH		5.45, br s		5.50, br s
3-NH		7.73, t (5.5)		7.78, t (5.5)
1′	97.1, CH	4.62, d (3.5)	97.0, CH	4.61, d (3.5)
2′	53.4, CH	3.67, dd	53.4, CH	3.67, dd
		(8.5, 3.5)		(8.5, 3.5)
3′	70.8, CH	3.44, dd	70.7, CH	3.41, dd
		(10.0, 8.5)		(10.0, 8.5)
4′	70.4, CH	3.16, ddd	70.4, CH	3.16, ddd
		(10.0, 9.0, 2.5)		(10.0, 9.0, 2.5)
5′	72.6, CH	3.39, ddd	72.7, CH	3.37, ddd
		(9.0, 5.0, 2.5)		(9.0, 5.0, 2.5)
6′	60.6, CH_2	3.58, m	60.5, CH_2	3.57, m,
		3.47, m		3.46, m
7′	169.3, C		169.2, C	
8′	22.8, CH_3	1.85, s	22.6, CH_3	1.85, s
2′-NH		7.52, d (8.5)		7.60, d (8.5)
3′-OH		4.78, br s		4.78, br s
4′-OH		5.00, br s		5.00, br s
6′-OH		4.45, dd		4.45, dd
		(12.0, 3.5)		(12.0, 3.5)

[a]^{1}H and ^{13}C data were recorded at 600 and 125 MHz, respectively.

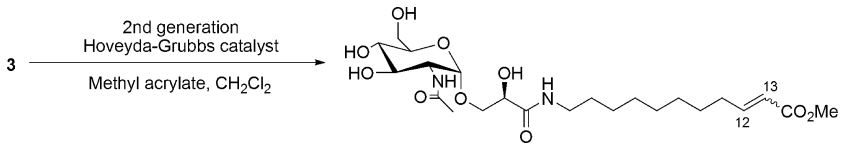

Figure 2. Olefin cross-metathesis of deinococcucin C (3).

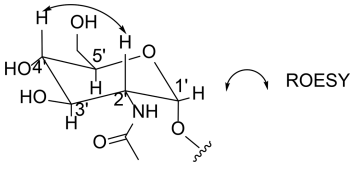

Figure 3. Strong ROESY correlations observed in the hexose moiety of 1.

$\Delta\delta_{S-R}$ values established the absolute configuration as 2R (Figure 4).

The biological activities of deinococcucins A−D (1−4) were evaluated in several ways. Deinococcucins A−D (1−4) were neither cytotoxic against human cancer cell lines (IC_{50} > 10 μM) nor active against Gram-positive and -negative bacteria or fungi (MIC > 128 μM) (see the Supporting Information).

Cancer chemoprevention is considered to play an important role in decreasing the risk of cancer development, involving the prevention, delay, or reversal of carcinogenesis. One important cancer prevention strategies is to enhance the deactivation of radicals and electrophiles via phase II enzymes.[33] Quinone reductase (QR), a representative phase II detoxification enzyme, is known to function as a cancer chemopreventative.[34] The QR activity in Hepa 1c1c7 cells was enhanced in a dose-dependent manner over a concentration range of 2.5−20 μM deinococcucins A (1) and C (3) without causing cytotoxicity.

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Deinococcucin C (**3**) induced QR activity by 1.8-, 2.2-, 2.5-, and 2.7-fold, and deinococcucin A (**1**) induced significant QR activity by 1.2-, 1.3-, 1.6-, and 1.9-fold at concentrations of 2.5, 5, 10, and 20 μM, respectively, as shown in Figure S29. However, no significant effects of deinococcucins B (**2**) and D (**4**) were observed (Figure S29).

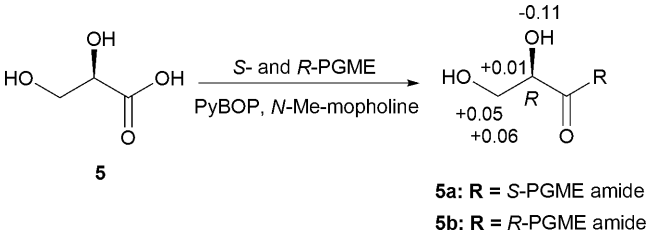

Figure 4. Degradation of **1** to 2,3-dihydroxypropanoic acid (**5**) and derivatization of **5** to *S*- and *R*-PGME amides. $\Delta\delta_{S-R}$ values of **5a** and **5b** are noted in ppm in DMSO-d_6.

Reports on the chemistry of the genus *Deinococcus* are sparse. From the representative species *D. radiodurans*, a radiation-resistant species deinoxanthin was reported as a red pigment possibly responsible for the antioxidant activity of this species against hydroxy radicals produced by photochemical reactions.[35] The other class of compounds from *D. radiodurans* consists of phosphoglycolipids.[36,37] The deinococcucins are structurally most similar to the glucosamine-containing phosphoglycolipids, of which the gross structures were proposed in mixtures without rigorous spectroscopic analysis or biological evaluation.[37] Our report of the new amino-glycolipids deinococcucins A–D is the first full characterization of glycolipids, including the determination of the exact double-bond position and the absolute configuration, from the genus *Deinococcus*. The similar structural features of the phosphoglycolipids from *D. radiodurans* and of the deinococcucins from the *Deinococcus* sp. that is most closely related to *D. xinjiangensis*, which is a phylogenetically distant species from *D. radiodurans* (Figure S28), indicate that the backbone of the deinococcucins could be a basic structural backbone of glycolipids in this genus.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco P-1020 polarimeter. IR spectra were recorded using a Thermo Nicolet iS10 spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were obtained on a Bruker Avance 600 MHz spectrometer at the National Center for Inter-University Research Facilities at Seoul National University (NCIRF). Low-resolution electrospray ionization source mass spectra were acquired with an Agilent Technologies 6130 quadrupole mass spectrometer coupled with an Agilent Technologies 1200 series high-performance liquid chromatography (HPLC) instrument. HRESIMS were acquired using a Thermo Scientific Q high-resolution mass spectrometer at the National Instrumentation Center for Environmental Management at Seoul National University. Semipreparative HPLC separations were achieved with a Gilson 305 pump and a Shodex 101 RI detector.

Collection of Carpenter Ants. Ant specimens were collected around a pond called Bandoji at the Seoul Zoo in Seoul Grand Park, Gwacheon-si, Gyeonggi-do, in the mating flight season (April 2015). Ant nests under rocks were excavated by a spade and a short half-moon hoe. Collected specimens were identified as carpenter ant *Camponotus japonicus* (Hymenoptera: Formicidae) in the field based on external morphological characteristics. These collected ants were divided into several containers using brushes and tweezers and transferred to the laboratory at Seoul National University on the same day.

Isolation and Classification of Strain SJN 1. Sixty queen ant specimens were washed with sterilized water and then soaked in 97% ethanol to remove the microbes on their exoskeleton. The queen ants were cut into three parts (head, thorax, and abdomen) using a razor blade. The cuticle of the abdomen was removed, and the intestinal parts were placed in a conical tube containing 20 mL of sterilized distilled water at 25 °C. The tube was vortexed, and 300 μL aliquots of the suspension were spread onto the surfaces of actinomycete isolation medium (1 L of distilled water, 22 g of actinomycete isolation medium, 18 g of agar, and 100 mg/L cycloheximide), A4 medium (1 L of distilled water, 18 g of agar, and 100 mg/L cycloheximide), A6 medium (1 L of distilled water, 18 g of agar, and 5 mg/L polymyxin B sulfate), A7 medium (1 L of distilled water, 18 g of agar, and 5 mg/L kanamycin), and chitin-based agar medium (1 L of distilled water, 4 g of chitin, 18 g of agar, and 100 mg/L cycloheximide) plates using autoclaved foam plugs (2 cm in diameter) and spreaders. The plates were incubated at 25 °C for 3 weeks. Strain SJN1 was isolated from a chitin-based agar plate. Colonies were repeatedly inoculated onto fresh YEME agar (1 L of distilled water, 18 g of agar, 4 g of yeast extract, 4 g of glucose, and 10 g of malt extract) plates to obtain a pure culture. Strain SJN1 was identified as a *Deinococcus* sp. most closely related to *D. xinjiangensis* based on its 16S rDNA sequence. (GenBank accession number: MF102142). Analysis of 16S rDNA sequences constructed a neighbor-joining phylogenetic tree to show the relationship between isolate SJN1 and the type strains of closely related species of the genus *Deinococcus* (Figure S28).

Cultivation and Isolation. The strain was cultivated in 50 mL of modified K medium (2 g of yeast, 2 g of glucose, 3 g of mannitol, 5 g of soytone, 5 g of starch, and 1 g of calcium carbonate in 1 L of distilled H_2O). After culturing the strain for 3 days on a rotary shaker at 180 rpm and 30 °C, 10 mL of the culture was inoculated into 1 L of modified K medium in a 2.8 L Fernbach flask and incubated at 180 rpm and 30 °C for 7 days. The entire culture (80 L) was extracted twice with ethyl acetate (EtOAc) and then concentrated *in vacuo* to yield 5 g of dry material. After extraction, the extract was fractionated by C_{18} reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as the eluent (five fractions eluted with an MeOH/H_2O gradient from 20% to 100%). After fractionation, deinococcucins A–D (**1**–**4**) were found in the 80% and 100% MeOH/H_2O fractions. Each fraction was then subjected to reversed-phase HPLC (250 × 10 mm Kromasil 100-5-C_{18} column, flow rate of 2 mL/min, RI detection, isocratic elution with 70% aqueous acetonitrile). Each compound was further purified (250 × 10 mm Kromasil 100-5-C_{18} column, flow rate of 2 mL/min, RI detection, isocratic elution with 82% aqueous MeOH) to yield deinococcucins A–D (**1**–**4**) (20, 15, 10, and 10 mg with yields of 0.25, 0.18, 0.13, 0.13 mg/L) at retention times of 29, 55, 31, and 76 min, respectively.

*Deinococcucin A (**1**):* colorless oil; $[\alpha]^{25}_D$ −25.0 (*c* 0.5, MeOH); IR (neat) ν_{max} 3360, 1640 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 1; ESIMS *m*/*z* 533.3756 [M + H]$^+$ (calcd for $C_{27}H_{53}N_2O_8$, *m*/*z* 533.3753).

*Deinococcucin B (**2**):* colorless oil; $[\alpha]^{25}_D$ −25.0 (*c* 0.5, MeOH); IR (neat) ν_{max} 3280, 1690 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 1; ESIMS *m*/*z* 547.3946 [M + H]$^+$ (calcd for $C_{28}H_{55}N_2O_8$, *m*/*z* 547.3943).

*Deinococcucin C (**3**):* colorless oil; $[\alpha]^{25}_D$ −17.0 (*c* 0.5, MeOH); IR (neat) ν_{max} 3300, 1630 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 2; ESIMS *m*/*z* 531.3650 [M + H]$^+$ (calcd for $C_{27}H_{51}N_2O_8$, *m*/*z* 531.3649).

*Deinococcucin D (**4**):* colorless oil; $[\alpha]^{25}_D$ −35.0 (*c* 0.5, MeOH); IR (neat) ν_{max} 3350, 1640 cm^{-1}; ^{1}H and ^{13}C NMR data in Table 2; ESIMS *m*/*z* 545.3750 [M + H]$^+$ (calcd for $C_{28}H_{53}N_2O_8$, *m*/*z* 545.3748).

Determination of the Double-Bond Position in Deinococcucins C (3) and D (4). A 0.5 mg amount of deinococcucin C (**3**) or D (**4**) was dissolved in a 10:1 mixture of CH_2Cl_2 and methyl acrylate (0.5 mL) to which 50 μg (5 mol %) of second-generation Hoveyda–Grubbs catalyst was subsequently added. The reaction mixture was

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stirred at room temperature for 2 h and injected into the LC/MS system without any treatment (100 × 4.6 mm Phenomenex C_{18} (2) column, gradient elution from 10% to 100% aqueous acetonitrile with 0.1% formic acid over 20 min and 100% acetonitrile with 0.1% formic acid after 20 min). The metathesis products of **3** and **4** commonly yielded an $[M + H]^+$ ion at m/z 505 at a retention time of 9.2 min in the LC/MS analysis. The double-bond positions of **3** and **4** were determined using the previously reported method based on the mass spectrometric data.[27]

Analysis of the Absolute Configuration of *N*-Acetyl Glucosamine in Deinococcucin A (1). Deinococcucin A (3 mg) was dissolved in 3 N HCl (0.5 mL) and stirred at 80 °C for 2 h. After the solution had cooled to rt, the HCl was evaporated *in vacuo* to yield the hydrolysate that contained glucosamine originating from *N*-acetyl glucosamine. HMDS and TMS-Cl (50 μL, v/v = 2:1) were added to the hydrolysate in pyridine (0.5 mL). After being stirred at 60 °C for 30 min, the mixture was dried *in vacuo* and separated using H_2O and CH_2Cl_2 (1 mL, v/v = 1:1). The CH_2Cl_2 layer was injected into a gas chromatograph equipped with an HP5 column (0.32 mm × 30 m). The injector and detector temperatures were maintained at 200 °C. During the analysis, the temperature of the GC column was controlled as follows: 60 °C for 3 min, 60−200 °C at 4 °C/min, and 200 °C for 3 min. The glucosamine derivative from the hydrolysate of **1** was detected at a retention time of 32.77 min. Authentic D-glucosamine and L-glucosamine samples were treated and analyzed using the same procedures. The derivatives of D-glucosamine and L-glucosamine were detected at 32.77 and 32.85 min, respectively. Co-injection of the silylated derivative of the hydrolysate and authentic D-glucosamine gave a single peak at 32.76 min, thereby determining the absolute configuration of the glucosamine in **1** as the D-form.

Acid Hydrolysis of Deinococcucin A (1). Deinococcucin A (**1**) (5.5 mg) was hydrolyzed in 6 N HCl (0.5 mL) at 40 °C. After 2 h, the reaction vial was cooled in ice water for 3 min. Then, the HCl was removed *in vacuo*, and the dry material was resuspended in 0.5 mL of H_2O and dried three times to completely remove the residual HCl. The reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was chromatographed over normal-phase silica gel (Waters Sep-Pak Vac 6 cm^3) using a solvent composed of a 30:1 mixture of $CHCl_3$/MeOH to yield the 2,3-dihydroxypropanoic acid (**5**, 2.0 mg).

*2,3-Dihydroxypropanoic acid (**5**):* ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.5 (s, OH), 6.1 (s, OH), 4.9 (s, OH), 4.47 (t, J = 5.0, 1H), 4.04 (dd, J = 12.5, 5.5, 1H), and 3.72 (dd, J = 12.5, 5.5, 1H).

PGME Amide Derivatization of 2,3-Dihydroxypropanoic Acid (5). First, 2 mg of 2,3-dihydroxypropanoic acid (**5**) from deinococcucin A (**1**) was dried under high vacuum for 24 h and dissolved in 500 μL of dimethylformamide (DMF). To the DMF solution of 2,3-dihydroxypropanoic acid (**5**) and *S*- or *R*-PGME were added benzotriazolyloxytris[pyrrolidino]phosphonium hexafluorophosphate (PyBOP) (4.4 mg, 8.4 μmol) and *N*-methylmorpholine (100 μL) at room temperature. After stirring for 1 h, a 5% HCl solution (1 mL) and EtOAc (2 mL) were added to the reaction mixture. The EtOAc layer was washed with saturated $NaHCO_3$ solution and brine. The *S*- and *R*-PGME amide products ($[M + H]^+$ m/z 254) were observed in the LC/MS analysis (100 × 4.6 mm, 5 μm, Phenomenex C_{18} (2) column; gradient elution from 10% to 100% aqueous acetonitrile with 0.1% formic acid over 20 min). After the reaction mixture was dried, the *S*- and *R*-PGME amide products (**5a** and **5b**) were purified by reversed-phase HPLC (250 × 10 mm, 5 μm, Kromasil C_{18} column; flow rate of 2 mL/min; UV detection at 254 nm; 50−100% aqueous acetonitrile gradient), eluting at retention times of 28 and 30 min, respectively.

*(S)-PGME amide of 2,3-dihydroxypropanoic acid (**5a**):* ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.58 (s, NH), 7.29−7.24 (m, 5H), 5.63 (s, 1H), 5.11 (s, OH), 4.54 (t, J = 6.5, 1H), 3.95 (dd, J = 12.0, 7.5, 1H), 3.88 (s, OH), 3.73 (s, 3H), and 3.58 (dd, J = 12.0, 7.5, 1H).

*(R)-PGME amide of 2,3-dihydroxypropanoic acid (**5b**):* ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.60 (s, NH), 7.31−7.26 (m, 5H), 5.61 (s, 1H), 5.22 (s, OH), 4.53 (t, J = 6.5, 1H), 3.90 (dd, J = 12.0, 7.5, 1H), 3.87 (s, OH), 3.71 (s, 3H), and 3.52 (dd, J = 12.0, 7.5, 1H).

QR Assays. The QR activities were determined spectrophotometrically using a previously reported modified microtiter method with murine Hepa-1c1c7 cells.[38] In this assay, 0.1% dimethyl sulfoxide (DMSO) was used as a negative control and β-naphthoflavone (2 μM) was used as a positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00426.

NMR spectra for **1**−**5** and phylogenetic tree of the strain SJN 1 (PDF)

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Notes

The authors declare no competing financial interest.

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