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Naphthoquinone−Oxindole Alkaloids, Coprisidins A and B, from a Gut-Associated Bacterium in the Dung Beetle, *Copris tripartitus*

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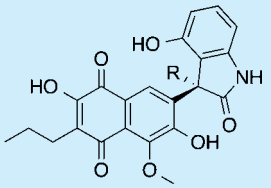
Supporting Information

ABSTRACT: Coprisidins A and B (**1** and **2**) were isolated from a gut-associated *Streptomyces* sp. in the dung beetle *Copris tripartitus*. Using a combination of spectroscopic techniques, the structures of the compounds were determined to be the first examples of natural naphthoquinone−oxindole alkaloids. Coprisidin A was found to inhibit the action of Na^+/K^+-ATPase, and coprisidin B showed activity for the induction of NAD(P)H:quinone oxidoreductase 1.



1: R = H
2: R = OH

Recent evidence suggests that insect-microbial symbioses are ubiquitous phenomena in insect ecology.[1] Meanwhile, the structural novelty and biological activity of metabolites produced by insect-associated microbes that play important roles by mediating symbioses encouraged the study of microorganisms in insect ecosystems as chemical sources for biomedical purposes.[2] Bioactive metabolites from insect-associated microbes are increasingly being reported. A new terpenoid with the botryane skeleton was discovered from the fungus *Hypocrea* sp. isolated from the insect *Serrataspis* sp.[3] A highly modified ansamycin was reported from a *Streptomyces* strain associated with the South African termite *Macrotermes natalensis*.[4] Our previous studies indicated that symbiotic bacteria in the dung beetle (*Copris tripartitus*) ecosystem are also a promising source of novel organic compounds.[5−8] For example, by examining dung beetle associated actinomycete strains, we have discovered tripartin, a dichlorinated indanone acting as a specific histone demethylase inhibitor,[5] and branched cyclic peptides coprisamides A and B, which act as inductors of quinone reductase.[6] In our continuing efforts since 2012 to search for microbial symbionts from the dung beetle *Copris tripartitus* inhabiting Jeju Island, we isolated a bacterial strain, SNU607, belonging to the genus *Streptomyces*, from the gut of *C. tripartitus*. Based on the analysis of 16S rDNA, the phylogenetically identical strains were isolated consistently from the guts of dung beetle specimens collected in 2013, 2014, and 2016, indicating that this particular bacterial strain may be tightly associated with the beetle. During our LC/MS (liquid chromatography/mass spectrometry) chemical analyses of these identical strains, we discovered that these strains, including SNU607, commonly produce a couple of previously unknown compounds that show distinct UV spectra along with a polyene macrocyclic lactam such as sceliphrolactam that was previously discovered from a mud dauber associated actinomycete.[9] Subsequent scaled-up culture enabled the further study of these unknown compounds and elucidation of their structures as naphthoquinone−oxindole alkaloids with a novel carbon framework. Here, we report the structures and biological activities of these structurally unique alkaloids, coprisidins A and B (**1** and **2**).

Coprisidin A (**1**) was obtained as a yellow powder. Its molecular formula was determined by high-resolution FAB mass spectrometry ($[M + H]^+ m/z$ 410.1242, calcd 410.1240) as $C_{22}H_{19}NO_7$ requiring 14 degrees of unsaturation. Interpretation of ^1H, ^{13}C, and HSQC NMR spectral data of **1** (Table 1) in DMSO-d_6 found four exchangeable protons (δ_H 10.86, 10.60, 9.80, and 8.81) due to NH and OH groups and four aromatic protons and carbons [δ_H 7.47, δ_C 120.4; δ_H 7.32, δ_C 133.9; δ_H 6.85, δ_C 113.9; δ_H 6.81, δ_C 115.2], indicating the features of an aromatic compound. In addition to these resonances, signals from three carbonyls (δ_C 183.7, 179.7, and 170.3), 10 quaternary carbons (δ_C 155.5, 155.3, 154.1, 149.3, 147.2, 131.0, 124.6, 123.6, 122.3, and 116.4), one methoxy (δ_H 3.77, δ_C 61.5), two methylene (δ_H 2.38, δ_C 24.8; δ_H 1.41, δ_C 21.2), and one methyl group (δ_H 0.88, δ_C 14.1) were observed.

In the COSY spectrum of **1**, we observed $^1H-^1H$ correlations of aromatic protons from H-6′ (δ_H 7.32) to H-5′ and H-7′ (δ_H 6.85 and δ_H 6.81) connecting the aromatic carbons C-5′, C-6′, and C-7′ (δ_C 113.9, δ_C 133.9, and δ_C 115.2). The vicinal coupling constants in this spin system (J = 7.5 Hz) strongly indicated that these protons belong to a 6-membered aromatic ring. As expected, the HMBC correlations from H-6′ to C-4′ and C-7′a (δ_C 155.3 and δ_C 149.3), from H-5′ to C-3′a (δ_C 116.4) and C-7′, and from H-7′ (δ_H 6.81) to C-3′a and C-5′ led to the identification of a six-membered ring. A

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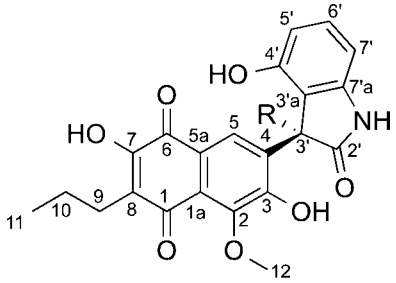
Table 1. NMR Data for Coprisidin A (1) in DMSO-d_6[a]

no.	δ_H	mult (J in Hz)	δ_C	type
1			183.7	C
1a			123.6	C
2			147.2	C
3			155.5	C
3-OH	10.86	s		OH
4			131.0	C
5	7.47	s	120.4	CH
5a			122.3	C
6			179.7	C
7			154.1	C
7-OH	10.60	br s		OH
8			124.6	C
9	2.38	t (7.0)	24.8	CH_2
10	1.41	qt (7.0, 7.0)	21.2	CH_2
11	0.88	t (7.0)	14.1	CH_3
12	3.77	s	61.5	CH_3
NH	8.81	s		NH
2′			170.3	C
3′	6.00	s	53.8	CH
3′a			116.4	C
4′			155.3	C
4′-OH	9.80	s		OH
5′	6.85	d (7.5)	113.9	CH
6′	7.32	dd (7.5, 7.5)	133.9	CH
7′	6.81	d (7.5)	115.2	CH
7′a			149.3	C

[a] ^{1}H and ^{13}C NMR were recorded at 600 and 150 MHz, respectively.



hydroxy group was assigned at C-4′ based on the HMBC correlations of 4′-OH (δ_H 9.80) to C-3′a, C-4′, and C-5′. The methine proton H-3′ displayed HMBC correlations to C-3′a, C-4′, and C-7′a in the six-membered aromatic ring, connecting C-3′ adjacent to C-3′a. In the HMBC spectrum, H-3′ was also correlated with the carbonyl carbon C-2′ with the chemical shift δ_C of 170.3, indicating amide or ester functionality. Together with the IR absorption at 1683 cm^{-1}, an exchangeable proton (δ_H 8.81) showed an HMBC correlation to this carbonyl carbon, assigning C-2′ as an amide functional group and the exchangeable proton as an amide NH. This amide NH also displayed 2,3-bond heteronuclear couplings with C-3′, C-3′a, C-7′a, and C-7′. These correlations assigned the amide NH directly next to C-7′a, closing a five-membered lactam ring and thus constructing an oxindole moiety.

A propyl group was easily assembled by ^{1}H−^{1}H COSY correlations of H_2-10 (δ_H 1.41) with H_2-9 (δ_H 2.38) and H_3-11 (δ_H 0.88). HMBC correlations from H_2-9 methylene to C-1, C-7, and C-8 (δ_C 183.7, δ_C 154.1, and δ_C 124.6) and from an exchangeable proton (δ_H 10.60) to C-6 (δ_C 179.7), C-7, and C-8 suggested the presence of two α,β-unsaturated ketones C-1 and C-6. 2,3-Bond heteronuclear correlations from another hydroxy proton (3-OH, δ_H 10.86) to C-2, C-3, and C-4 (δ_C 147.2, δ_C 155.5, and δ_C 131.0) indicated that this aromatic alcohol group is attached to C-3. Furthermore, a singlet aromatic proton, H-5 (δ_H 7.47), showed strong three-bond heteronuclear couplings with three aromatic carbons, C-1a, C-3, and C-6, revealing a naphthoquinone partial structure. A methoxy group was assigned to C-2 based on the H_3-12 (δ_H 3.77)/C-2 correlation in the HMBC spectrum. Finally, these oxindole and naphthoquinone moieties were connected by the HMBC correlations from H-3′ to C-4 and C-5 (δ_C 120.4) and from H-5 to C-3′. Hence, the planar structure of coprisidin A (**1**) was determined as a structurally new naphthoquinone−oxindole satisfying the 14 degrees of unsaturation (Figure 1).

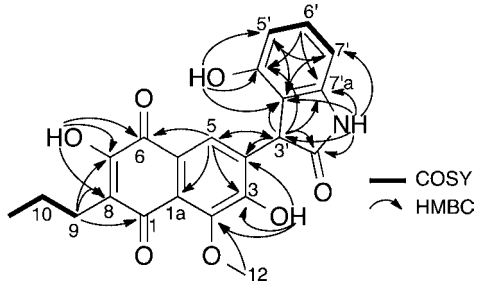


Figure 1. Key COSY and HMBC correlations of coprisidin A (**1**).

Coprisidin B (**2**) was acquired as a yellow powder. Its molecular formula was evaluated by high-resolution FAB mass spectrometry ([M + H]$^+$$m/z$ 426.1191, calcd 426.1189) as $C_{22}H_{19}NO_8$. Careful comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** indicated a high degree of similarity to coprisidin A (**1**). The difference between **1** and **2** was due to the replacement of the methine proton at C-3′ in **1** by a hydroxy group, 3′-OH (δ_H 7.17) in **2**, as confirmed by the combinational analysis of 2D NMR data (Table S1 and Figures S7−S12).

For the analysis of the absolute configuration of C-3′ in **1**, a conformational search for **1** was performed using MacroModel with the Merck Molecular Force Field (gas phase), a 2 kJ/mol upper energy limit, and 0.001 kJ (mol Å)$^{-1}$ convergence threshold on the rms gradient to minimize computational complexity and expense. An energy-minimized structure of each conformer was calculated (Figures 2 and Figure S15).[10] Then the ECD (electronic circular dichroism) spectra of the conformers of the two enantiomers (3′*S* and 3′*R*) of **1** were

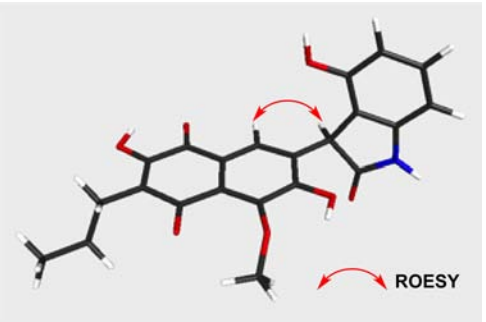


Figure 2. Energy-minimized structure of the major conformer of coprisidin A (**1**) with the 3′*S* configuration.

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calculated by time-dependent density-functional theory (TD-DFT) at the B3LYP/def2-TZVPP//B3LYP/def-SV(P) level for all atoms.[11] The final calculated ECD spectra were obtained by Boltzmann averaging of all of the ECD spectra of the conformers (Table S35).[10] The experimental ECD spectrum of **1** displayed a positive Cotton effect at 275 to 325 nm originated from the interactions between the two chromophores, naphthoquinone and oxindole moieties. The calculated ECD spectrum of the enantiomer with the 3′*S* configuration was consistent with the experimental ECD spectrum (Figure 3 and Table S2−9), whereas the other enantiomer (*ent*-compound **1** with 3′*R*) showed the opposite ECD spectrum, indicating that coprisidin A possesses the 3′*S* configuration. The absolute configuration at the C-3′ position in **2** was also proposed as *R* by the comparison of the experimental ECD spectrum and the ECD spectra of **2** calculated by the same procedure as in **1** (Figures S16−18).

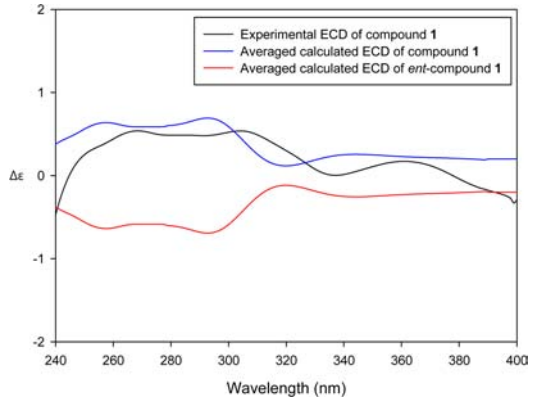

Figure 3. Comparison of the experimental ECD spectrum of **1** with the averaged calculated ECD spectra.

To evaluate the biological activity of coprisidins A and B (**1** and **2**), we first tested the compounds in cell-based cytotoxicity assays against different target cancer cell lines (HCT 116, A549, SNU-638, SK-HEP-1, and MDA-MB-231); however, no significant cytotoxicity was observed. After a series of biological activity studies for the coprisidins, we found that coprisidin A (**1**) inhibited the action of Na^+/K^+-ATPase (IC_{50} = 21.8 μM) while coprisidin B (**2**) showed a far weaker inhibition (IC_{50} = 200 μM). Coprisidin B also inhibited the action of *S. aureus* derived sortase A (IC_{50} = 77.5 μM).

During our continuing search for bioactivity, we observed that only coprisidin B showed activity for induction of the NQO1. NAD(P)H:quinone oxidoreductase 1 (NQO1), a phase II detoxifying enzyme which plays an important role in cancer prevention, and the induction of this enzyme is a potential target for the prevention of carcinogenesis.[12] To evaluate the effect of coprisidin B (**2**) on NQO1 activity, various concentrations of coprisidin B (2.5, 10, and 40 μM) were treated in cultured Hepa 1c1c7 cells for 24 h. As shown in Figure 4a, coprisidin B (**2**) induced the NQO1 activity in a concentration-dependent manner, while coprisidin A (**1**) did not display a significant inducting effect. The induction of the enzyme activity by coprisidin B was found to be associated with the up-regulation of NQO1 protein expression. In addition, coprisidin B showed the up-regulation of heme oxygenase-1 (HO-1) expression, another phase II enzyme (Figure 4b).

To the best of our knowledge, coprisidins A and B are the first naphthoquinone−oxindole alkaloids as natural products.

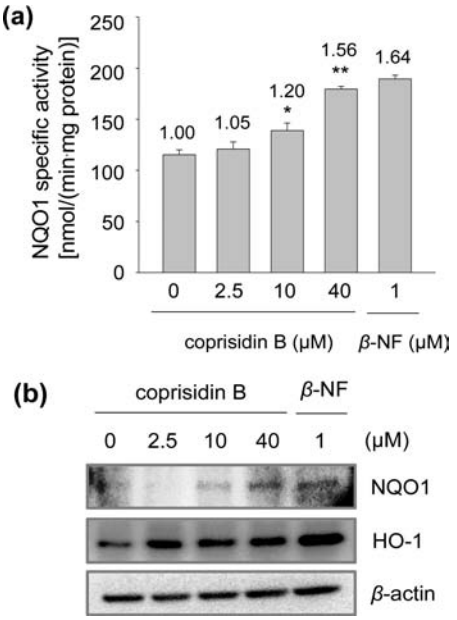

Figure 4. Effects of coprisidin B on NQO1 activity. (a) Hepa 1c1c7 cells were treated with indicated concentrations of coprisidin B and *β*-naphthoflavone (*β*-NF), a positive control at 37 °C for 24 h (see the Supporting Information). Values represent the mean ± SD of four determinations. *$p < 0.05$, **$p < 0.001$ indicate statistically significant differences from the control group. (b) Effects of coprisidin B on the expression of NQO1 and HO-1. The cells were treated with coprisidin B for 24 h, and then protein expressions were analyzed by Western blotting.

Several synthetic naphthoquinone−oxindole compounds were previously reported.[13] However, the carbon skeletons of these synthetic compounds are significantly different by connecting the two moieties at their quinone rings whereas the coprisidins tether the naphthoquinone and oxindole moieties at their benzene rings. Although the oxindole structure is relatively common in fungi, it is rarely reported in actinomycetes. Recent actinomycete-originated oxindole representatives are prenylated oxindoles from *Actinoplanes missouriensis*[14] and *Streptomyces* sp.[15]

The formation of the naphthoquinone−oxindole backbone in the coprisidins is biosynthetically intriguing. A ready hypothetical biosynthesis could be via a hybrid pathway composed of nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) involving a tryptophan building unit as proposed for the fungal metabolites codinaeopsin[16] and cyclopiazonic acid.[17] However, feeding L-tryptophan-1-^{13}C in bacterial cultivation, which was intended to incorporate ^{13}C at C-2 in **1** and **2** and thus provide supportive evidence for the hypothesis, did not result in any incorporation of the ^{13}C-labled precursor into the coprisidins (Figure S21). In the experiment, the production levels of **1** and **2** were not noticeably affected by the precursor feeding. This result could not support the hypothetical NRPS-PKS hybrid pathway using tryptophan itself as a starting unit.

A carbon−carbon bond formation between a naphthoquinone and a tryptophan-derived indole via a radical laccase-type coupling[18] could be an alternative hypothesis. However, feeding L-tryptophan-indole-2-^{13}C to the bacterial strain, which aimed to label C-1′ in **1** and **2**, did not incorporate a ^{13}C-labeled oxindole moiety into these compounds (Figure S22). This result does not support the hypothesis that the oxindole ring

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could be originated from a tryptophan and possibly couples with a naphthoquinone to yield the coprisidins. The naphthoquinone moiety, if it is biosynthesized independently from the oxindole, could be putatively originated from a PKS pathway similar to that reported in fungi.[19] An additional feeding experiment with pentanoic acid-1-^{13}C, which could be a PKS pathway starting unit for the naphthoquinone moiety in the coprisidins, decreased the production of coprisidin A by 45% without any incorporation of pentanoic acid into the molecules. Thus, the feeding experiment did not support the role of pentanoic acid as the hypothetical PKS starter (Figure S23). Overall, despite our feeding experiments using ^{13}C-labeled putative precursors, the biosynthetic pathway of the coprisidins is still unclear. Therefore, further extensive genetic and biochemical studies are required to elucidate the biosynthesis of the coprisidins.

In summary, naphthoquinone−oxindole alkaloids, coprisidins A and B, were discovered from a gut-associated *Streptomyces* sp. strain that was consistently isolated in the dung beetle *C. tripartitus* over four years. Coprisidins A and B were determined to be the first instance of natural naphthoquinone−oxindole alkaloids to the best of our knowledge. Considering the structural novelty and biological activity of the coprisidins, our discovery accentuates the chemical diversity from insect gut associated bacterial communities and their great potential as a source of new bioactive compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02555.

Detailed experimental procedures and NMR data for compounds **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Douglas, A. E. *Cell Host Microbe* **2011**, *10*, 359−367. (b) Dossey, A. T. *Nat. Prod. Rep.* **2010**, *27*, 1737−1757. (c) Oh, D.-C.; Poulsen, M.; Currie, C. R.; Clardy, J. *Nat. Chem. Biol.* **2009**, *5*, 391−393. (d) Scott, J. J.; Oh, D.-C.; Yuceer, M. C.; Klepzig, K. D.; Clardy, J.; Currie, C. R. *Science* **2008**, *322*, 63.

(2) (a) Crawford, J. M.; Clardy, J. *Chem. Commun.* **2011**, *47*, 7559−7566. (b) Cragg, G. M.; Newman, D. J. *Biochim. Biophys. Acta, Gen. Subj.* **2013**, *1830*, 3670−3695. (c) Choi, H.; Oh, D.-C. *Arch. Pharmacal Res.* **2015**, *38*, 1591−1605.

(3) Yuan, Y.; Feng, Y.; Ren, F.; Niu, S.; Liu, X.; Che, Y. *Org. Lett.* **2013**, *15*, 6050−6053.

(4) Kim, K. H.; Ramadhar, T. R.; Beemelmanns, C.; Cao, S.; Poulsen, M.; Currie, C. R.; Clardy, J. *Chem. Sci.* **2014**, *5*, 4333−4338.

(5) Kim, S.-H.; Kwon, S. H.; Park, S.-H.; Lee, J. K.; Bang, H.-S.; Nam, S.-J.; Kwon, H. C.; Shin, J.; Oh, D.-C. *Org. Lett.* **2013**, *15*, 1834−1837.

(6) Um, S.; Park, S. H.; Kim, J.; Park, H. J.; Ko, K.; Bang, H.-S.; Lee, S. K.; Shin, J.; Oh, D.-C. *Org. Lett.* **2015**, *17*, 1272−1275.

(7) Um, S.; Bang, H.-S.; Shin, J.; Oh, D.-C. *Nat. Prod. Sci.* **2013**, *19*, 71−75.

(8) Kim, S.-H.; Ko, H.; Bang, H.-S.; Park, S.-H.; Kim, D.-G.; Kwon, H. C.; Kim, S. Y.; Shin, J.; Oh, D.-C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5715−5718.

(9) (a) Oh, D.-C.; Poulsen, M.; Currie, C. R.; Clardy, J. *Org. Lett.* **2011**, *13*, 752−755. (b) Poulsen, M.; Oh, D.-C.; Clardy, J.; Currie, C. R. *PLoS One* **2011**, *6*, e16763.

(10) Smith, S. G.; Goodman, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 12946−12959.

(11) Bringmann, G.; Tasler, S.; Endress, H.; Kraus, J.; Messer, K.; Wohlfarth, M.; Lobin, W. *J. Am. Chem. Soc.* **2001**, *123*, 2703−2711.

(12) Chen, C.; Kong, A. N. *Free Radical Biol. Med.* **2004**, *36*, 1505−1516.

(13) (a) Wu, D.; Zhang, X.; Xu, Y.; Xue, Y.; Li, J.; Wang, W.; Zhu, J. *Asian J. Org. Chem.* **2014**, *3*, 480−486. (b) Yu, J.-S.; Zhou, F.; Liu, Y.-L.; Zhou, J. *Beilstein J. Org. Chem.* **2012**, *8*, 1360−1365.

(14) Satou, R.; Izumikawa, M.; Katsuyama, Y.; Matsui, M.; Takagi, M.; Shin-ya, K.; Ohnishi, Y. *J. Antibiot.* **2014**, *67*, 231−236.

(15) Jang, J.-P.; Nogawa, T.; Uramoto, M.; Okano, A.; Futamura, Y.; Shimizu, T.; Takahashi, S.; Jang, J.-H.; Ahn, J. S.; Osada, H. *J. Antibiot.* **2015**, *68*, 293−295.

(16) Kontnik, R.; Clardy, J. *Org. Lett.* **2008**, *10*, 4149−4151.

(17) Liu, X.; Walsh, C. T. *Biochemistry* **2009**, *48*, 8746−8757.

(18) Ševčík, V. *Nature* **1957**, *179*, 966−967.

(19) Kim, J.-E.; Kim, J.-C.; Jin, J.; Yun, S.-H.; Lee, Y.-W. *Plant Pathol. J.* **2008**, *24*, 8−16.

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