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Streptoxamine, an unprecedented benzoisoindole-deferoxamine hybrid from the locust-derived *Streptomyces* sp. HKHCa2

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ABSTRACT

An unusual benzoisoindole-deferoxamine hybrid, streptoxamine (**1**), has been isolated from the ethyl acetate crude extract of the fermentation broth of a locust-associated actinomycete, *Streptomyces* sp. HKHCa2, which was isolated from an insect, *Oxya chinensis*. The structure of this secondary metabolite was elucidated on the basis of its one-dimension, two-dimension NMR, and mass spectroscopic data. This natural product features a hybrid pattern of a benzoisoindole with an "iron carrier" deferoxamine B through C-N linkage. Compound **1** showed weak antibacterial activity against the gram-positive bacteria, *Staphylococcus aureus* and *Mycobacterium smegmatis*.

1. Introduction

Insects, possessing the most rich groups, are often associated with symbiotic microorganisms which provide nutrition, immunological regulators, antibiotics, *etc.* to support their host survival and adaptation [1,2]. In the insect-symbiont associations, the host organisms often harbor specific microbial symbionts [3], providing a tremendous discovery opportunity for isolating new species of bacteria and fungi. Explorations of the microbes in symbiotic insect ecosystems can give new insights into the natural products discovery and have led to the discovery of plentiful of bioactive natural products with novel chemical scaffolds. For example, a series of immunosuppressive polyketides with different novel carbon skeletons were reported from the fungus *Daldinia eschscholzii* IFB-TL01 isolated from the gut of the mantis species *Tenodera aridifolia* [4,5]. Bacterial symbionts with insect are also a promising source of novel bioactive natural products and a great many bioactive metabolites from actinomycete are being reported such as the Na^+/K^+-ATPase inhibitor, coprisidin A, and NAD(P)H:quinone oxidoreductase inducer, coprisidin B from a dung beetle (*Copris tripartitus*)-associated *Streptomyces* strain [6], and antimicrobial 20-membered glycosylated macrolactams, macrotermycins A-D from a termite-associated *Amycolatopsis* sp. M39 [7]. In addition, *Deinococcus* sp. from the carpenter ant *Camponotus japonicus* and *Microbacterium* sp. from the carrion beetle *Nicrophorus concolor* produced novel aminoglycolipids deinococcucins A-D and chlorinated cyclic peptides nicrophorusamides A and B, respectively [8,9]. Our previous chemical investigation of insect-associated actinomycetes also led to the discovery of chemically intriguing bioactive molecules like novel enediyne-derived compounds amycolamycins A and B, and unusual macrolactams rifamorpholines A-E from the locust (*Locusta migratoria*)-associated rare actinobacterium *Amycolatopsis* sp. Hca4, and tetrasaccharide derivatives actinotetraoses A–H and angucyclines from *Amycolatopsis* sp. HCa1 derived from the locust, *Oxya chinensis* [10–14]. In our continuing efforts to search for insect-associated bacterial symbionts, recently we isolated an actinomycete strain *Streptomyces* sp. HKHCa2 from a healthy insect *Oxya chinensis*, collected from Haikou, People's Republic of China. Subsequent chemical study on the large-scale fermentation broth by a combination of column chromatography led to the isolation and identification of an unusual benzoisoindole-deferoxamine hybrid, streptoxamine (**1**) (Fig. 1). This compound features a hybrid pattern of a benzoisoindole with an "iron carrier" deferoxamine B through C-N linkage and showed weak antibacterial activity against the gram-positive bacteria, *Staphylococcus aureus* and *Mycobacterium smegmatis*. Herein, we report the isolation and structure elucidation of this novel compound, as well as its antibacterial activity.

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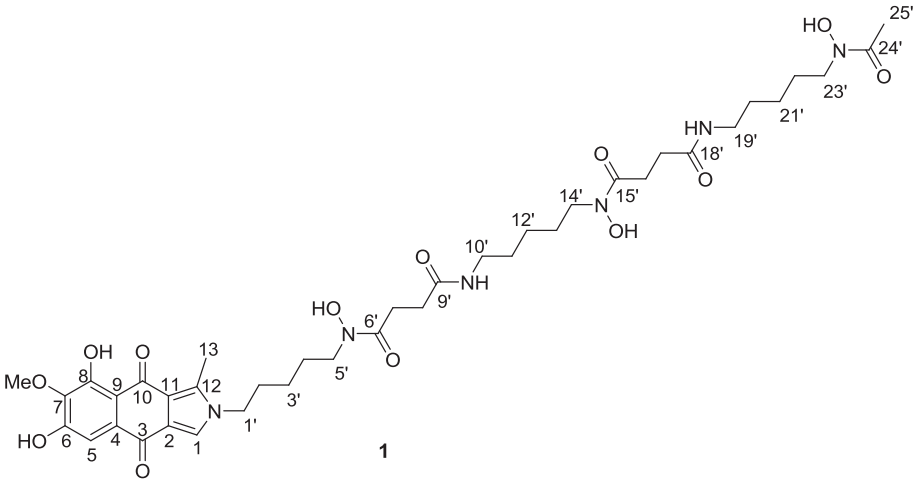

Fig. 1. Structure of streptoxamine (**1**).

2. Results and discussion

Streptoxamine (**1**) was isolated as a yellow oil with the molecular formula of $C_{39}H_{56}N_6O_{13}$ derived from the high-resolution electrospray ionization mass spectrum (HR-ESI-MS) and ^{13}C NMR data (Table 1). The ^{13}C NMR and DEPT135 spectra of **1** displayed signals of thirty nine carbons, including seven carbonyl carbons (δ_C 186.1–170.6), two aromatic/olefinic methine carbons (δ_C 126.1, 108.8), eight non-protonated aromatic/olefinic carbons (δ_C 157.8–111.5), two methyl carbons (δ_C 20.8, 11.3), one oxygenated methyl carbons (δ_C 60.3), and nineteen aliphatic methylene carbons (δ_C 47.5–23.4). The ^1H NMR spectrum of **1** in DMSO-d_6 exhibited signals of two methyls at δ_H 2.59 (3H, s, H-13) and 1.95 (3H, s, H-25′), one oxygenated methyls at δ_H 3.79 (3H, s, 7-OMe), two aromatic protons [δ_H 7.63 (1H, s, H-1) and 7.08 (1H, s, H-5)], nineteen aliphatic methylenes (δ_H 3.96–1.20), two hydroxyl groups at δ_H 13.62 (1H, br s, 8-OH) and 9.63 (1H, br s, 6-OH), and two NH groups at δ_H 7.76 (1H, t, J = 5.6, 10′-NH) and 7.74 (1H, t, J = 5.6, 19′-NH). These data above showed that **1** could contain three other *N*-hydroxyl moieties and three rings.

The complete assignments for all protons and carbons, as shown in Table 1, were deduced by comprehensive analysis of HSQC, ^1H-^1H COSY, HMBC, and ROESY spectra (Fig. 2). In **1**, The naphthoquinone substructure could be identified by the observation of HMBC correlations from 8-OH to C-7 (δ_C 139.3), C-8 (δ_C 157.8) and C-9 (δ_C 111.5), from 7-OMe to C-7, from H-5 to C-3 (δ_C 178.5), C-4 (δ_C 131.7), C-6 (δ_C 157.8), C-7 and C-9, from H-1 to C-2 (δ_C 120.8), C-3 and C-11 (δ_C 117.2), from H_3-13 to C-11 and C-12 (δ_C 137.8). The ^1H,^1H three-bond couplings observed in the COSY spectrum from H_2-2′ to H_2-1′ and H_2-3′, from H_2-4′ to H_2-3′ and H_2-5′, from H_2-11′ to H_2-10′ and H_2-12′, from H_2-13′ to H_2-12′ and H_2-14′, from 10′-NH to H_2-10′, from H_2-20′ to H_2-19′ and H_2-21′, from H_2-22′ to H_2-21′ and H_2-23′, from 19′-NH to H_2-19′, together with the chemical shifts of the ^{13}C resonances (C-1′, C-5′, C-10′, C-14′, C-19′ and C-23′) observed at relative higher field (δ_C 47.5–38.9), revealed the presence of three mono-*N*-hydroxylated diaminopentane moieties [15]. ^1H-^1H COSY correlations from H_2-7′ (δ_H 2.25, m) to H_2-8′ (δ_H 2.56, m) and from H_2-16′ (δ_H 2.25, m) to H_2-17′ (δ_H 2.56, m) and HMBC correlations from H_2-7′ to C-9′ (δ_C 171.7), from H_2-8′ to C-6′ (δ_C 172.4), from H_2-17′ to C-15′ (δ_C 172.4) and from H_2-16′ to C-18′ (δ_C 171.7) identified two unsymmetrical succinyl residues in **1**.

In the HMBC spectrum, correlations from H_2-5′ (δ_H 3.47, t, J = 7.0) to C-6′, from 10′-NH to C-9′ and C-10′ (δ_C 38.9), from H_2-10′ (δ_H 2.99, m) to C-9′, from H_2-14′ to C-15′, from 19′-NH to C-18′ and C-19′ (δ_C 38.9), and from H_2-19′ (δ_H 2.99, m) to C-18′ suggested that each of the two succinyl residues was linked to two pentanediamine fragments *via* amide bonds. An acetyl group was connected with the 23′-*N*-hydoxyl on the basis of the HMBC correlations from H_2-23′ (δ_H 3.44, t, J = 7.0) and H_3-25′ to C-15′ (δ_C 170.6). These deductions above were also supported by the ROESY correlations from 10′-NH to H_2-8′, H_2-10′ and H_2-11′, from 19′-NH to H_2-17′, H_2-19′ and H_2-20′. The key HMBC correlations observed from H_2-1′ (δ_H 3.96, t, J = 7.0) to C-1 and C-12 suggested that the nitrogen on the pentanediamine fragment was linked to C-1 and C-12 on the naphthoquinone substructure, thus forming the last ring and completing the structure of **1**. This connectivity was also secured by the observation of the ROESY correlations from H-1 and H_3-13 to H_2-1′ and H_2-2′. Therefore, the complete structure of streptoxamine (**1**) was determined as shown in Fig. 1. This unusual natural product features a hybrid pattern of a naphthoquinone with an “iron carrier” deferoxamine B through C-N linkage, which could be generated by non-enzymatic condensation of the terminal amino group in deferoxamine B with the isofurano moiety with the same strategy for pyridine ring biosynthesis [16,17].

Compound **1** was assessed for antibacterial activity against selected Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium smegmatis*. As a result, it only displayed weak antibacterial activity

Table 1
^1H (700 MHz) and ^{13}C NMR (175 MHz) spectroscopic data for streptoxamine (**1**) in DMSO-d_6.

Position	δ_H (mult, J in Hz)	δ_C	Position	δ_H (mult, J in Hz)	δ_C
1	7.63, s	126.1, CH	7′	2.25, m	30.3, CH_2
2		120.8, C	8′	2.56, m	28.0, CH_2
3		178.5, C	9′		171.7, C
4		131.7, C	10′	2.99, m	38.9, CH_2
5	7.08, s	108.8, CH	10′-NH	7.76, t (J = 5.6)	
6		157.8, C	11′	1.37, m	29.3, CH_2
6-OH	9.63, br s		12′	1.20, m	24.0, CH_2
7		139.3, C	13′	1.49, m	26.5, CH_2
7-OMe	3.79, s	60.3, CH_3	14′	3.44, t (J = 7.0)	47.5, CH_2
8		157.8, C	15′		172.4, C
8-OH	13.62, s		16′	2.25, m	30.3, CH_2
9		111.5, C	17′	2.56, m	28.0, CH_2
10		186.1, C	18′		171.7, C
11		117.2, C	19′	2.99, m	38.9, CH_2
12		137.8, C	19′-NH	7.74, t (J = 6.0)	
13	2.59, s	11.3, CH_3	20′	1.37, m	29.3, CH_2
1′	3.96, t (J = 7.0)	46.9, CH_2	21′	1.20, m	24.0, CH_2
2′	1.71, p (J = 7.0)	29.8, CH_2	22′	1.49, m	26.5, CH_2
3′	1.23, m	23.4, CH_2	23′	3.44, t (J = 7.0)	47.2, CH_2
4′	1.54, p (J = 7.0)	26.2, CH_2	24′		170.6, C
5′	3.47, t (J = 7.0)	47.2, CH_2	25′	1.95, s	20.8, CH_3
6′		172.4, C			