ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal

# Si-enterobactin from the endophytic *Streptomyces* sp. KT-S1-B5 – a potential silicon transporter in Nature?†

Cite this: DOI: 10.1039/c3cc44437f

Received 13th June 2013,
Accepted 28th June 2013

DOI: 10.1039/c3cc44437f

www.rsc.org/chemcomm

Timothée J. N. Kenla,[a] Michel D. Kongue Tatong,[a] Ferdinand Mouafo Talontsi,[a] Birger Dittrich,[b] Holm Frauendorf[a] and Hartmut Laatsch*[a]



**Si-enterobactin (2a), a hexacoordinated complex of the siderophore enterobactin (2b) with silicon as the central atom, was isolated from an endophytic *Streptomyces* sp. occurring in *Piper guinensis* roots. The structure and absolute configuration were determined from NMR and MS data, and by X-ray diffraction. The orientation of the molecule along the pseudo-3-fold axis shows that the coordination environment of the silicon atom complexed with three bidentate ligands is *Δ*. We assume that 2a or related complexes may be involved in the transport of silicon in plants, diatoms, or other silicon-dependent organisms.**

As part of our ongoing search for novel bioactive secondary metabolites from endophytes inhabiting Cameroonian medicinal plants,[1] we investigated a *Streptomyces* sp., strain KT-S1-B5, isolated from the roots of *Piper guinensis.* Metabolic profiling by HPLC-UV-MS/MS, and subsequent dereplication by $^1$H NMR using AntiBase,[2] delivered six known metabolites: *N*-acetylglucosamine, hopene B, aggreceride B, methyl 13-methyltetradecanoate, and the siderophores nocardamine[3] and desoxynocardamine.[4] In addition, evidence obtained *via* HPLC/ESIHRMS indicated the presence of protochelin[5] and ferrioxamine E (see Fig. S7 in ESI†). A further siderophore, enterobactin (**2b**), was also present, but was isolated as the unusual orthosilicate **2a** instead of the expected iron complex. It was obtained in crystalline form after chromatographic separation, but was also found in the crude extract by ESIMS before further work-up, if the fermentation was performed in glass vials or better in the presence of traces of silica gel. In this communication, we report the isolation, structure elucidation and absolute configuration of **2a**, referred to hereafter as Si-enterobactin.

The $^1$H NMR spectrum (see Table S1 and Fig. S2, ESI†) of **2a** showed three aromatic protons in the 1,2,3-positions, an oxy-methylene group and a methine signal. In addition, an exchangeable NH signal was observed at $\delta_H$ 10.17 (d, $J$ = 10.5 Hz). The $^{13}$C NMR data (Table S1 and Fig. S3 in ESI†) confirmed this interpretation. Of the ten $^{13}$C NMR signals observed, the two at $\delta_C$ 169.5 and 167.2 had values indicative of acid derivatives. From the 2D NMR data (see Fig. S4 and S5 in ESI†), an *N*-(2,3-dihydroxybenzoyl)-serine subunit **1** was elucidated (Fig. 1). This unit is known to occur in the free state in *E. coli*,[6] but is more important as a substructure of the iron-transporting salmochelins[7] or in the cyclotrimer of **1**, enterobactin (**2b**).[8,9]

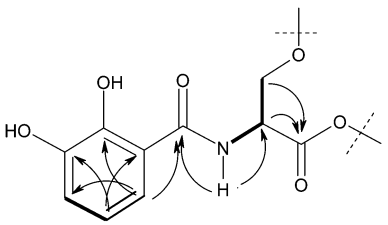
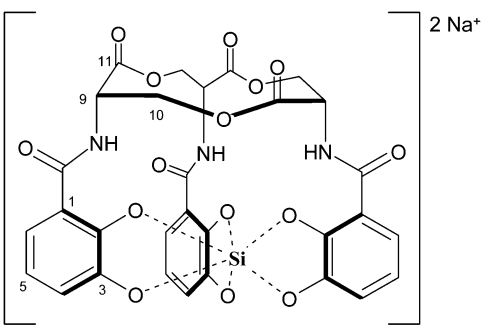



**Fig. 1** Selected $^1$H–$^1$H COSY and HMBC correlations of sub-structure **1**.

The mass spectra displayed several pseudomolecular ions, but their masses (*m*/*z* 345, 692, 714 at (−)-ESI; 391, 694, 716, 738, 760 at (+)-ESI) did not match the monomeric or oligomeric catechol siderophores of type **1**/**2b** or their iron complexes. However, the HRMS data did agree fairly well with the aluminium complex of enterobactin (**2b**), and very well with its silicon derivative (for assignments see the Experimental part).




**2a**

**2b**: 6 OH instead of $O_6Si$

[a] *Institute of Organic and Biomolecular Chemistry, University of Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany. E-mail: hlaatsc@gwdg.de*
[b] *Institute for Inorganic and Applied Chemistry, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany*

† Electronic supplementary information (ESI) available: $^1$H, $^{13}$C, and 2D NMR spectra (COSY, HSQC and HMBC), HRESI MS, and crystallographic data of Si-enterobactin (**2a**). CCDC 920703. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc44437f

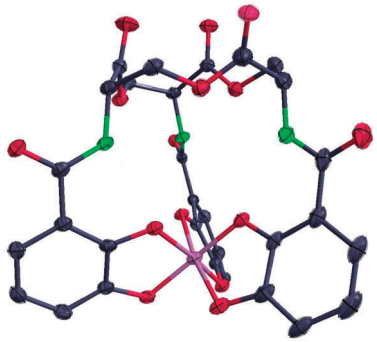


**Fig. 2** ORTEP plot (ellipsoids at 50% probability) of the anisotropic displacements of Si-enterobactin (**2a**). Hydrogen and methanol atoms have been omitted for clarity.

X-ray diffraction of single crystals grown from a methanolic solution confirmed the orthosilicate **2a** (Fig. 2). The structure was solved by direct methods in the $P2_1$ space group and refined with the 2012 version of SHELXL.[10] The presence of silicon was unambiguously established using the bond-valence method,[11] where the charge of the central atom is calculated from the six bond distances observed. Further confirmation of silicon as the central atom was obtained from a $^{29}$Si NMR spectrum, which showed a singlet at $\delta$ −140.5 (see ESI,† Fig. S6). With **2b**, we solved the first crystal structure of an organic silicon complex isolated from Nature.

Determination of the absolute structure of the molecule was also possible. The refinement of the Flack parameter[12] gave a value of 0.018(8) and confirmed the absolute structure and the (*S*) configuration of all three serine residues.[13] The orientation of the molecule along the pseudo-3-fold axis showed that the coordination environment of the silicon atom complexed with the three bidentate ligands is *Λ* (Tables S8 and S10, ESI†). With respect to the protonation state of the oxygen atoms of the catechol ligands, no hydrogen atoms were found, giving a di-anion. While some residual electron density remains in the octahedral environment on all six bonds between the oxygens and the silicon atom, none of these peaks can be assigned to refinable hydrogen atoms. The charge of the catechol entities is instead balanced by two proximate sodium cations, which are further coordinated by the (partly disordered) methanol solvent. Si–O bond distances are 1.780(2), 1.786(2), 1.789(2), 1.793(2), 1.793(2), and 1.796(2) Å, indicating that the presence of hydrogen atoms can be excluded with confidence (Table S10, ESI†).

Enterobactin (**2b**), first isolated from *Salmonella typhimurium*,[8,9] is known to form stable iron complexes, but the hexacoordinated complex **2a** from a streptomycete is the first low-molecular weight organosilicate isolated from Nature. It is noteworthy that we have detected **2a** and **2b** using (−)-ESI MS already after fermentation in Erlenmeyer flasks before a chromatographic work-up (Fig. S16, ESI†), although the yield of **2a** was higher in the presence of traces of silica gel; in the presence of soil samples, however, **2a**, **2b**, or nocardamine were not formed. In separate experiments, enterobactin (**2b**) did not dissolve silica gel or diatomaceous earth at neutral pH during formation of **2a**, and therefore a formation during work-up is unlikely. However, under slightly basic conditions, **2a** was formed quantitatively from **2b** in the presence of silica gel at ambient temperature, and a sandy soil sample formed the Si complex even without addition of a base (pH 7.01 in an aqueous suspension). This is not in contrast to our fermentation experiment in the presence of soil, as iron compounds present there are known to suppress the formation of siderophores. We conclude that enterobactin can incorporate silicate in the basic fermentation broth from glass or – in the natural habitat – soil. This reaction occurs nearly quantitatively, as the free **2b** was detected as a trace component only in a few of our fermentations under Si limitation (Fig. S16 and also for further results, see ESI†).

Nevertheless, the formation of **2b** is not completely unexpected. Recently, Süssmuth and co-workers synthesized a number of **2b** complexes, among them also **2a**.[14] They found that the triscatechol derivative **2b** is capable of binding silicic acid to form **2a** at physiological pH, when cultivating *E. coli* in the presence of sodium silicate. Other synthetic catechol derivatives showed a similar behaviour.[15]

In contrast to the occurrence of silicon in bacteria, its importance for plants, diatoms and certain other organisms like glass sponges is very well established.[16,17] Generally, the silicon concentration is relatively high in plant tissues, and in some cases can exceed the concentration of nitrogen and potassium,[18] reaching up to 10% of the dry mass.[16] Horsetails belonging to the family *Equisetaceae*, as well as diatoms and glass sponges, require silicon as an essential nutrient.[16,19] Silicon enhances growth in plants, and protects them from abiotic and biotic stresses such as infections by fungal pathogens and insects. In addition, it enhances the physical stability of plants, and is a cell wall substitute in diatoms.[20]

The uptake of silicon occurs in plants *via* neutral silicic acid by means of transporter proteins of the aquaporin family (water channel proteins) that transport it to the shoot epidermis. Here it polymerizes to biogenic amorphous silica.[21] Diatoms use functionally related proteins. The concentration of soluble silicic acid or silicate in the soil is moderate (0.1–0.6 mM) and often growth limiting.[18] Therefore, it can be speculated that Si-enterobactin (**2a**) or related compounds of microbial origin are involved synergistically in the transport of biological silicon *via* mobilization of silicate, perhaps by means of associated or endophytic microorganisms. The application of Si–catechol complexes as growth stimulators has already been tried, but was not very successful due to their low stability.[22] In contrast, Si-enterobactin is remarkably stable, and survives in boiling water for more than 30 minutes. Removal of the silicon atom, whilst keeping the organic ligand intact, was achieved *via* a short treatment with hydrofluoric acid at room temperature; in this way, the **2b** required for our previously discussed experiments was generated. Due to its stability, supplementation of the soil with Si-enterobactin or **2a**-producing bacteria may have positive effects on plant growth. In particular, such supplementation may increase the harvest of silicon-demanding plants like rice. It may also be of interest to search for **2a** or related low-molecular weight Si transporters in the tissue or the rhizosphere of Si-rich plants. Solutions of **2a** may reach rather high silicon molarities of theoretically up to 2 M for silicic acid (as calculated for solid **2a**, using molecular weight and density data; see ESI,† Table S1),

without losing solubility. In contrast, free silicic acid starts to polymerize into silica at concentrations >2 mM, although the intracellular concentration of silicic acid may reach values of up to 340 mM. Compounds related to **2a** are therefore good candidates for the expected organic chelators maintaining supersaturated solutions in the Si pools of diatoms and other organisms.[23]

For general experimental procedures, see ref. 1.

Using our previously described methods,[1] the endophytic *Streptomyces* sp. KT-S1-B5 was isolated from surface-sterilized roots of *Piper guinensis* (Piperaceae), a Cameroonian medicinal plant collected in Mbouda, Cameroon, in May 2008. The isolate was identified as *Streptomyces* sp. by microbial techniques. The strain KT-S1-B5 is preserved in our microbial collection at the Institute of Organic and Biomolecular Chemistry, Georg-August University of Göttingen.

For fermentation, extraction and isolation, see ESI.†

Si-enterobactin (**2a**): colorless monoclinic crystals from MeOH. (For $^{1}$H and $^{13}$C NMR data, see Table S1 and Fig. S1–S6, ESI.†) (−)-ESI HRMS: $m/z$ = 345.5379 $[M - 2H]^{2-}$, (calcd for $[C_{30}H_{21}N_3O_{15}Si]^{2-}$, 345.5381), 692.0830 $[M - H]^{-}$, (calcd for $[C_{30}H_{22}N_3O_{15}Si]^{-}$, 692.0527), 714.0645 $[M - 2H + Na]^{-}$, (calcd for $[C_{30}H_{21}N_3O_{15}Si\ Na]^{-}$, 714.0645); (+)-ESI HRMS: $m/z$ = 391.5 $[M - 2H + 4Na]^{+}$, (calcd for $[C_{30}H_{21}N_3O_{15}SiNa_4]^{2+}$, 391.51610), 694.0974 $[M + H]^{+}$, (calcd for $[C_{30}H_{24}N_3O_{15}Si]^{+}$, 694.0971), 716.0792 [M + Na], (calcd for $[C_{30}H_{23}N_3O_{15}Si\ Na]^{+}$, 716.0791), 738.0610 $[M - H + 2Na]^{+}$, (calcd for $[C_{30}H_{22}N_3O_{15}SiNa_2]^{+}$, 738.0610), 760.0432 $[M - 2H + 3Na]^{+}$, (calcd for $[C_{30}H_{21}N_3O_{15}SiNa_3]^{+}$, 760.0430).

For complexation experiments with **2b**, see ESI.†

An endophytic *Streptomyces* sp. isolated from the rhizosphere of *Piper guinensis* was found to produce Si-enterobactin (**2a**), the first natural organosilicate. The absolute configuration of the three serine residues was established as (*S*) on the basis of X-ray crystallography, while a $\Delta$ configuration was assigned to the central silicon atom. We assume that Si-enterobactin (**2a**) or related compounds synthesized by plant-associated or endophytic microorganisms are involved in the transport of silicon, an essential element for many plants. Our experiments have shown that **2a** is not an artefact generated during work-up, but a true natural product.

This work was supported by the German Academic Exchange Service (DAAD) by a grant to F.M.T. for PhD studies. B.D. thanks the Deutsche Forschungsgemeinschaft (DFG) for an Emmy-Noether fellowship (DI 921/3-2). We thank Mr R. Machinek and Dr M. John for the NMR spectra, Mrs F. Lissy and Mr A. Kohl for technical assistance, and Dr S. Hickford for language polishing. T.J.N.K., M.D.K.T., and F.T.M. thank the German Academic Exchange Service (DAAD) for fellowships.

## Notes and references

1 F. M. Talontsi, PhD thesis, University of Göttingen, 2011; F. M. Talontsi, Md. T. Islam, P. Facey, C. Douanla-Meli, A. von Tiedemann and H. Laatsch, *Phytochem. Lett.*, 2012, **5**, 657–664.
2 H. Laatsch, *AntiBase 2012, A Data Base for Rapid Dereplication and Structure Determination of Microbial Natural Products*, Wiley VCH, Weinheim, Germany, 2012.
3 S. Konetschny-Rapp, G. Jung, K. N. Raymond, J. Meiwes and H. Zahners, *J. Am. Chem. Soc.*, 1992, **114**, 2224–2230.
4 G.-Y.-S. Wang, E. Graziani, B. Waters, W. Pan, X. Li, J. McDermott, G. Meurer, G. Saxena, R. J. Andersen and J. Davies, *Org. Lett.*, 2000, **2**, 2401–2404; H.-S. Lee, H. J. Shin, K. H. Jang, T. S. Kim, K.-B. Oh and J. Shin, *J. Nat. Prod.*, 2005, **68**, 623–625.
5 K. Taraz, G. Ehlert, K. Geisen, H. Budzikievicz, H. Korth and G. Pulyerer, *Z. Naturforsch.*, 1990, **45b**, 1327–1332.
6 J. B. Neilands, in *Inorganic Biochem.*, ed. G. Eichhorn, *et al.*, Elsevier, Amsterdam, 1973, p. 167.
7 K. Hantke, G. Nicholson, W. Rabsch and G. Winkelmann, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3677–3682; B. Bister, D. Bischoff, G. J. Nicholson, M. Valdebenito, K. Schneider, G. Winkelmann, K. Hantke and R. D. Süssmuth, *BioMetals*, 2004, **17**, 471–481.
8 J. R. Pollack and J. B. Neilands, *Biochem. Biophys. Res. Commun.*, 1970, **38**, 989–992.
9 K. N. Raymond, E. A. Dertz and S. S. Kim, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3584–3588.
10 G. M. Sheldrick, *Acta Crystallogr., Sect. A*, 2008, **64**, 112–122, and private communications.
11 N. F. Brese and M. O'Keefe, *Acta Crystallogr., Sect. A*, 1991, **B47**, 192–197.
12 H. D. Flack, *Acta Crystallogr., Sect. A*, 1983, **39**, 876–881.
13 CCDC 920703.
14 T. Schmiederer, S. Rausch, M. Valdebenito, Y. Mantri, E. Mösker, T. Baramov, K. Stelmaszyk, P. Schmieder, D. Butz, S. I. Müller, K. Schneider, M.-H. Baik, K. Hantke and R. D. Süssmuth, *Angew. Chem., Int. Ed.*, 2011, **50**, 4230–4233.
15 S. Bai, Y. Tsuji, Y. Okaue and T. Yokoyama, *Chem. Lett.*, 2008, 1168–1169.
16 E. Epstein, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1999, **50**, 641–664.
17 J. F. Ma, N. Yamaji and N. Mitani-Ueno, *Proc. Jpn. Acad.*, 2011, **87B**, 377–385.
18 E. Epstein, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 11–17.
19 F. M. Hoffman and C. J. Hillson, *Bot. Gaz.*, 1979, **140**, 127–132.
20 V. Martin-Jézéquel, M. Hidebrand and M. A. Brzezinski, *J. Phycol.*, 2000, **36**, 821–840.
21 J. F. Ma, K. Tamai, N. Yamaji, N. Mitani, S. Konishi, M. Katsuhara, M. Ishiguro, Y. Murata and M. Yano, *Nature*, 2006, **440**, 688–691.
22 C. C. Perry and Y. Lu, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 2915–2921.
23 K. Thamatrakoln and M. Hildebrand, *Plant Physiol.*, 2008, **146**, 1397–1407.