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COMMUNICATION

A family of multicyclic indolosesquiterpenes from a bacterial endophyte†

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Three novel indolosesquiterpenes, xiamycin B (1b), indosespene (2), and sespenine (3), along with the known xiamycin A (1a) were isolated from the culture broth of *Streptomyces* sp. HKI0595, a bacterial endophyte of the widespread mangrove tree *Kandelia candel*. Agar diffusion assays revealed moderate to strong antimicrobial activities against several bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*, while no cytotoxicity against human tumor cell lines was observed. Together with the previously reported oridamycin, the endophyte metabolites represent the first indolosesquiterpenes isolated from prokaryotes.

Introduction

Indolosesquiterpenes represent a group of alkaloids with diverse biological activities, including antibiotics, antiparasitic agents, non-steroidal progestins, and inhibitors of lipid droplet biosynthesis. Interestingly, indolosesquiterpenes were primarily found in plants. Important examples are polyalthenol from *Polyalthia oliveri*,[1] 3-farnesylindoles from *Uvaria pandensis*,[2] isopolyalthenol, neopolyalthenol,[3] polyveoline,[4] polyavolinamide,[5] and polysin[6] from *Polyalthia suaveolens*. From *Greenwayodendron suaveolens*, suaveolindole,[7] greenwayodendrin-3-one[8] and pentacyclindole[9] were isolated. Only during the past decade, a few indolosesquiterpenes were discovered in fungi, namely sespendole[10] from *Pseudobotrytis terrestris*, as well as the lacanindoles from *Verticillium lecanii*.[11] Yet, indolosesquiterpenes from procaryotes have been unknown until recently, when we and others independently reported the structures of the bacterial carbazole alkaloids xiamycin[12] and oridamycin,[13] respectively. While the oridamycins were produced by a soil-dwelling actinomycete,[13] xiamycin has been found in the broth of an endophytic *Streptomyces* sp. isolated from the stem of the mangrove tree *Bruguiera gymnorrhiza*.[12] Here we report the discovery of three novel bacterial indolosesquiterpenes along with the known xiamycin as metabolites of an endophyte of another important mangrove plant, *Kandelia candel*.

Results and discussion

Kandelia candel is a widespread mangrove tree found southern Asia, such as in southern India, southeast China and southern Japan, yet information of its bacterial endophytes and their metabolites is scarce.[14,15] In a program to investigate bioactive natural products from bacterial mangrove endophytes,[12,16] we succeeded in the isolation of an endophytic *Streptomyces* sp. (strain HKI0595) from the stem of a *K. candel* sample, and a preliminary metabolic profiling indicated the production of various novel natural products. To elucidate their structures, we performed a large-scale fermentation (200 L), and the crude extract was subjected to open column chromatography on silica and size-exclusion chromatography, and repeated preparative HPLC, which eventually yielded **1a** (2.2 mg), **1b** (1.0 mg), **2** (2.5 mg), and **3** (14.2 mg) as pure compounds (Fig. 1).

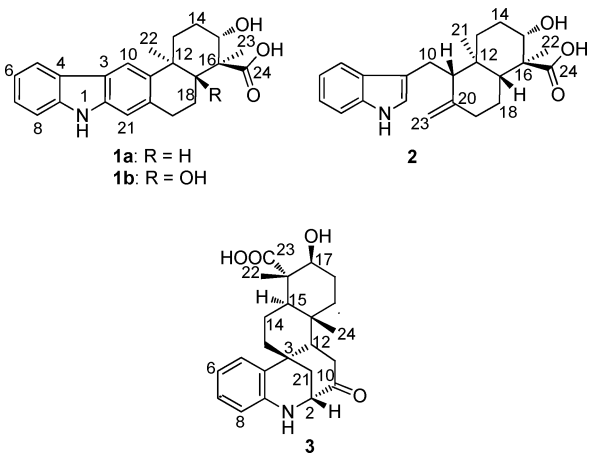

Fig. 1 Structures of xiamycin A (**1a**), xiamycin B (**1b**), indosespene (**2**), and sespenine (**3**).

Except for **1b**, which was only produced in minute amounts, all compounds were subjected to cytotoxicity and antimicrobial assays.[17] **1a**, **2**, and **3** did not inhibit proliferation of any of the tumor cell lines tested up to a concentration of 10 μM (**2**, **3**), or

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30 μM (**1a**) (ESI†). Using *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium vaccae*, methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* as test strains, **1a** exhibited the strongest antibacterial activities, while **2** and **3** showed only moderate or weak antibacterial activities, respectively (ESI†).

Through NMR and mass spectrometry, the structures of the four endophyte metabolites were elucidated, revealing that they belong to a small family of bacterial indolosesquiterpenes. First, we were startled to re-encounter xiamycin (**1a**), the previously discovered indolosesquiterpene metabolite from the endophyte of the mangrove plant *Bruguiera gymnorrhiza*.[12] All physicochemical data obtained were in full agreement with the ones we found for xiamycin. However, since **1b** represents a congener of **1a** (see below) we named the latter xiamycin A.

According to high resolution ESIMS and ^{13}C NMR data, **1b** has a molecular formula of $C_{23}H_{25}NO_4$ (*m/z* 378.1756 $[M - H]^-$). The UV spectrum of **1b** and its strong violet color reaction with anisaldehyde-sulfuric acid, which is typical for indole derivatives, already suggested that **1b** is a derivative of **1a**. In the aromatic region of the ^1H NMR spectrum of **1b**, four consecutive signals (δ 7.92, 7.38, 7.29, 7.08) from a 1,2-disubstituted benzene ring, along with two singlets (δ 7.48, 7.15) from a carbazole ring system were also observed.[18] H,H COSY correlations established partial structures of a 1,2-disubstituted benzene ring, CH_2-CH_2-CH-OH, and CH_2-CH_2, which were similar to xiamycin (**1a**). Apart from one additional oxygenated quaternary carbon signal (δ 79.7, C-17), ^{13}C NMR and DEPT spectra of **1b** showed high similarities to **1a**, revealing 12 sp^2 signals from the carbazole ring carbons. This finding was further supported by HMBC correlations (Fig. 2). Because of the congruent NMR signals, we concluded that **1b** features the same pentacyclic skeleton as **1a**. Furthermore, since the NOE was observed between H_a-18, Me-22 and Me-23 (Fig. 3), **1b** has the same overall configuration as **1a**. Finally, from the HMBC correlation of H-13 and Me-22 to C-17 we concluded that **1b** (xiamycin B) differs from **1a** only in the carbinol group at C-17.

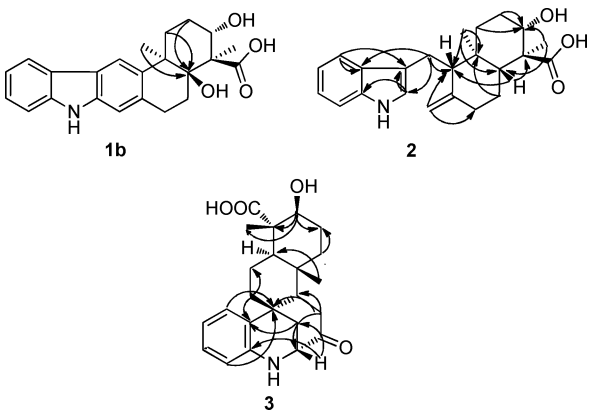

Fig. 2 Selected HMBC correlations for compounds **1b**, **2** and **3**.

The physicochemical data of **2** and its molecular formula $C_{23}H_{29}NO_3$ deduced from HRESIMS and ^{13}C NMR suggested that it represents an indolosesquiterpene variant lacking two double bond equivalents compared to **1a** and **1b**. All three compounds share similar NMR spectra, but in the aromatic region of the ^1H NMR spectrum of **2**, one singlet appeared at δ 6.89 (H-2) in place

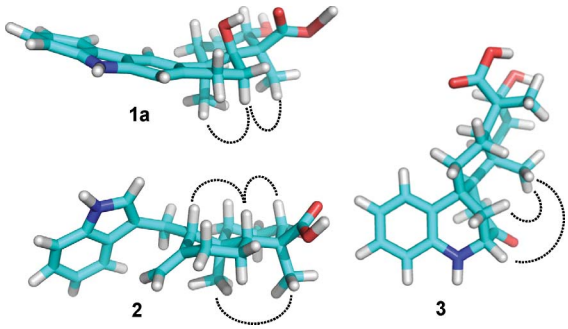

Fig. 3 Selected NOESY correlations for compounds **1b**, **2** and **3**.

of two singlets for **1a** and **1b**, which indicated that the carbazole ring was disrupted. However, HMBC correlations between H-2 and C-3 (δ 129.0) and C-4 (δ 115.6) showed that the indole ring was intact. Moreover, we deduced the structure of a bicyclic sesquiterpene skeleton from H,H COSY and HMBC correlations (Fig. 2). HMBC correlations between H-10 and C-2, C-3 and C-4 provided important information on the connection of the sesquiterpene portion to the indole ring. The relative configuration of **2** was inferred from the NOESY spectrum, where correlations between H-11, H-15 and H-17, as well as between Me-21 and Me-22 were observed (Fig. 3). Compound **2**, named indosespene, seems to be a precursor of **1a** and represents a novel member of the indolosesquiterpene family of natural products.

For compound **3**, the molecular formula of $C_{23}H_{29}NO_4$ was established through HRESIMS (*m/z* 384.2191, $[M + H]^+$) and ^{13}C NMR data. While its NMR spectra were similar to the ones for **1a**, **1b** and **2**, in the aromatic region only four consecutive signals (δ 7.28, 6.96, 6.63, 6.55) were visible that were diagnostic for a 1,2-disubstituted benzene ring. The ^{13}C NMR spectrum showed signals for two carbonyl groups (δ 212.0, 181.4), six aromatic carbons constituting the benzene ring, and another fifteen saturated carbons. These findings indicated that the framework of **3** largely differs from **1a**, **1b**, and **2**. ^1H NMR and H,H-COSY spectra revealed partial structures of NH-CH-CH_2, CO-CH_2-CH, and a terpenoid fragment as found for **1a** and **2**. The presence of the partially conserved terpene part was confirmed by HSQC and HMBC correlations (Fig. 2). HMBC correlations from H-2 (δ 3.64) to C-3 and C-9, from H-5 and H-8 to C-3 revealed the connection of the nitrogen heterocycle to the benzene part. Further correlations from H-11 to C-2 and C-3, and H-13 to C-4 fully established the remaining substructures of the terpene to give an unusual pentacyclic architecture. NOE effects between H-2 and Me-24, and Me-22 and Me-24 indicated the same configuration as in **1a**, **1b**, and **2**. Compound **3**, named sespenine, represents another rare bacterial indolosesquiterpene with a heavily rearranged skeleton.

Compounds **1a**, **1b**, **2** and **3** feature rare heterocyclic ring systems, and their co-occurrence in a bacterium is intriguing from a biosynthetic point of view. Notably, the only known natural products that are structurally related to **2** and **3** are the *Aspergillus nomius* metabolites nominine (**4**)[18] and aspernomine (**5**),[19] respectively. In this context it is interesting to point out a recent synthetic study in which the acid-promoted arrangement of a substituted 3*H*-indol-3-ol to an uncommon 1-benzazocin-3(2*H*)-one ring system was observed.[20] Based on these findings it was plausible to conclude that **2a** and **3a** as well as **2** and **3**

are biogenetically related. We reason that xiamycins, indosespene and sespenine share a farnesylindole progenitor, and that the side chain is first activated through epoxidation and then cyclized into the decaline system of indosespene. Oxidation of the indole ring would then set the stage for an anellation reaction, and the resulting carbenium would allow for two alternative reaction channels. Deprotonation, loss of an equivalent of water, and aromatization would yield the xiamycin ring system (Fig. 4, route a). Alternatively, the phenyl would migrate to the angular carbenium resulting in a disruption of the indole ring and formation of the pentacyclic ring system with a central quaternary carbon (Fig. 4, route b). This model would be in full agreement with the relative and absolute configurations observed for **1b**, **2**, **3**, and **1a**, respectively.

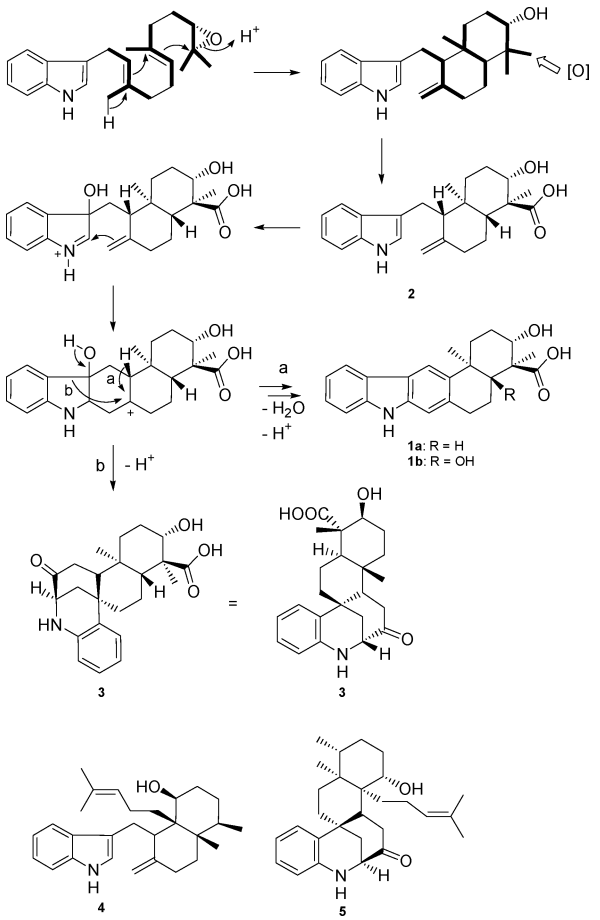

Fig. 4 Model for the biosynthesis of **1a**, **1b**, **2** and **3** from proposed precursor, 3-farnesylindole epoxide. Structures of nominine (**4**) and aspernomine (**5**).

In conclusion, we have investigated the metabolic capability of a bacterial endophyte of the mangrove tree *Kandelia candel* and isolated xiamycin A along with three new alkaloids, xiamycin B, indosepene and sespenine. While these compounds show no activity against human tumor cell lines, in agar diffusion tests **1a** and **2** proved to be active against several Gram-positive and Gram-negative bacteria, including multi-resistant ones. The newly discovered indosespene, sespenine, along with xiamycin and oridamycin, represent the first examples of indolosesquiterpenes isolated from bacteria. It is intriguing that these prokaryotes are endophytes producing typical plant metabolites. Furthermore, the structures and co-occurrence of the group of indolosesquiterpenes in a single strain suggest a biogenetic relationship, and on the basis of the structures a plausible biosynthetic model was established. Future studies at the molecular level may provide insights into yet scarcely studied indole-terpenoid biosynthesis in bacteria.

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References

1 M. Leboeuf, M. Hamonniere, A. Cave, H. E. Gottlieb, N. Kunesch and E. Wenkert, *Tetrahedron Lett.*, 1976, **17**, 3559.
2 M. H. H. Nkunya and H. Weenen, *Phytochemistry*, 1989, **28**, 2217.
3 N. Kunesch, A. Cave, M. Leboeuf, R. Hocquemiller, G. Dubois, E. Guittet and J. Y. Lallemand, *Tetrahedron Lett.*, 1985, **26**, 4937.
4 R. Hocquemiller, G. Dubois, M. Leboeuf, A. Cave, N. Kunesch, C. Riche and A. Chiaroni, *Tetrahedron Lett.*, 1981, **22**, 5057.
5 D. A. Okorie, *Phytochemistry*, 1981, **20**, 2575.
6 I. Ngantchou, B. Nyasse, C. Denier, C. Blonski, V. Hannaert and B. Schneider, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3495.
7 H. D. Yoo, P. A. Cremin, L. Zeng, E. Garo, C. T. Williams, C. M. Lee, M. G. Goering, M. O'Neil-Johnson, G. R. Eldridge and J. F. Hu, *J. Nat. Prod.*, 2005, **68**, 122.
8 C. M. Hasan, T. M. Healey, P. G. Waterman and C. H. Schwalbe, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2807.
9 R. B. Williams, J. F. Hu, K. M. Olson, V. L. Norman, M. G. Goering, M. O'Neil-Johnson, G. R. Eldridge and C. M. Starks, *J. Nat. Prod.*, 2010, **73**, 1008.
10 R. Uchida, Y. P. Kim, T. Nagamitsu, H. Tomoda and S. Omura, *J. Antibiot.*, 2006, **59**, 338.
11 D. M. Roll, L. R. Barbieri, R. Bigelis, L. A. McDonald, D. A. Arias, L. P. Chang, M. P. Singh, S. W. Luckman, T. J. Berrodin and M. R. Yudt, *J. Nat. Prod.*, 2009, **72**, 1944.
12 L. Ding, J. Münch, H. Goerls, A. Maier, H. H. Fiebig, W. H. Lin and C. Hertweck, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6685.
13 K. Takada, H. Kajiwara and N. Imamura, *J. Nat. Prod.*, 2010, **73**, 698.
14 S. H. Guan, I. Sattler, W. H. Lin, D. A. Guo and S. Grabley, *J. Nat. Prod.*, 2005, **68**, 1198.
15 S. H. Guan, S. Grabley, I. Groth, W. H. Lin, A. Christner, D. Guo and I. Sattler, *Magn. Reson. Chem.*, 2005, **43**, 1028.
16 L. Ding, A. Maier, H. H. Fiebig, H. Görls, W. H. Lin, G. Peschel and C. Hertweck, *Angew. Chem., Int. Ed.*, 2011, **50**, 1630.
17 W. A. Dengler, J. Schulte, D. P. Berger, R. Mertelsmann and H. H. Fiebig, *Anti-Cancer Drugs*, 1995, **6**, 522.
18 J. B. Gloer, B. L. Rinderknecht, D. T. Wicklow and P. F. Dowd, *J. Org. Chem.*, 1989, **54**, 2530.
19 G. M. Staub, J. B. Gloer, D. T. Wicklow and P. F. Dowd, *J. Am. Chem. Soc.*, 1992, **114**, 1015.
20 Y. Liu, W. W. J. McWhorter and C. E. Hadden, *Org. Lett.*, 2003, **5**, 333.