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Hybrid Isoprenoids from a Reeds Rhizosphere Soil Derived Actinomycete *Streptomyces* sp. CHQ-64

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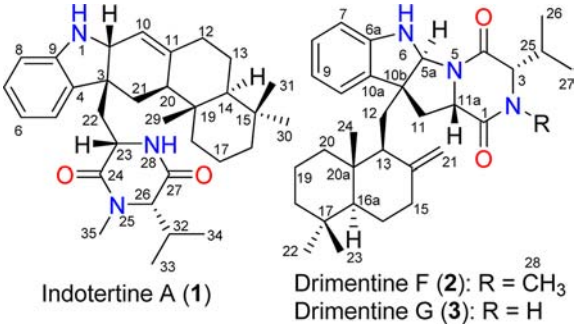
ABSTRACT



Indotertine A (1), a hybrid isoprenoid with a condensed pentacyclic skeleton, together with two related hybrid isoprenoids, drimentines F (2) and G (3), were isolated from a reeds rhizosphere soil derived actinomycete *Streptomyces* sp. CHQ-64. Their structures including absolute configurations were elucidated by spectroscopic methods, X-ray single crystal diffraction analysis, and TDDFT ECD calculations. Drimentines G (3) showed strong cytotoxicity against human cancer cells lines with IC_{50}'s down to 1.01 μM, while 1 and 2 showed no significant activity.

Hybrid isoprenoids (HIs) are biosynthesized by attaching terpenoid moieties of different levels of complexity to molecules produced *via* nonterpenoid biosynthetic routes.[1] HI production is quite rare among prokaryotes;[1,2] however, the reported HI metabolites of bacterial origin have interesting structural diversity and biological activities, including a number of important compounds such as novobiocin (DNA gyrase inhibitor),[3] nitropyrrolin (anticancer),[4] and neomarinonone (anticancer).[5]

During our ongoing search for structurally novel and bioactive natural products from microorganisms isolated from marine sediment, we investigated the metabolites of 103 marine-derived actinomycetes by integrated chemical and bioassay screening. Among them, strain CHQ64 identified as *Streptomyces* sp. (Genbank No.: JQ405211) isolated from reeds rhizosphere soil collected from the mangrove conservation area of Guangdong province, China, was selected for its significant cytotoxicity against P388 cells and interesting HPLC profile of the fermentation broth extract. Spectroscopic studies resulted in the isolation and identification of three new related hybrid isoprenoids, named indotertine A (**1**) and drimentines F (**2**)

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(1) Gallagher, K. A.; Fenical, W. *Curr. Opin. Biotechnol.* **2010**, *21*, 794–800.
(2) Kuzuyama, T.; Seto, H. *Nat. Prod. Rep.* **2003**, *20*, 171–183.
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and G (**3**).[6] Among them, indotertine A (**1**) has a novel scaffold characterized by the condensed ring system containing a tryptophane's indole moiety and a sesquiterpene unit and represents a new class of HIs merging the NRPS and mevalonate pathways.

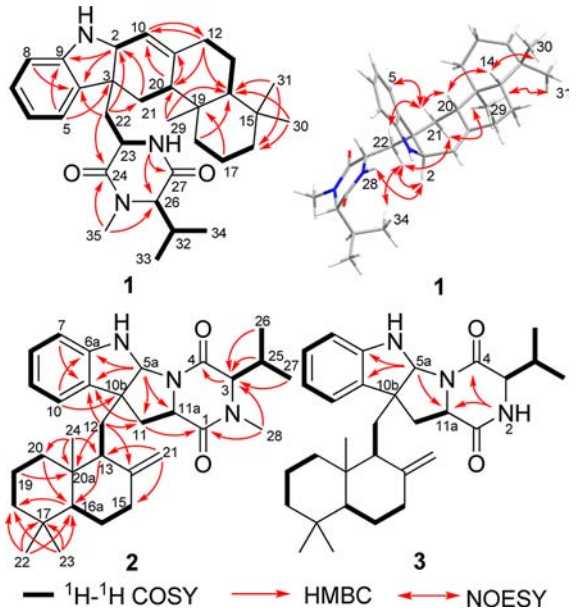

Figure 1. Selected 2D NMR correlations for compounds **1**–**3**.

The bacterium (strain CHQ64) was incubated on a rotatory shaker at 28 °C for 7 days and then harvested by extraction with EtOAc. The extract (35.5 g) was separated by repeated silica gel column chromatography followed by an LH-20 column and finally semiprep ODS HPLC to yield compounds **1**, **2**, and **3** (0.28, 0.48 and 0.56 yields, respectively) (Supporting Information (SI)).

Indotertine A (**1**)[7] was obtained as an optially active colorless amorphous powder. Its molecular formula was determined as $C_{32}H_{45}N_3O_2$ according to the HRESIMS at m/z 504.3583 $[M + H]^+$, requiring 12 degrees of unsaturation. The ^{1}H and ^{13}C NMR data of compound **1** displayed resonances that were assigned to 8 quaternary carbons, 11 methines, 7 methylenes, and 6 methyls (including one *N*-methyl) (Table S4, SI). The sesquitepene moiety and diketopiperazine unit composed of tryptophan and N-Me valine were deduced by the interpretation of ^{1}H−^{1}H COSY and HMBC spectra readily, and the planar structure of **1** was further constructed by the ^{1}H−^{1}H COSY (H-2/H-10) and HMBC correlations from H-21 to C-2 and from H-22 to C-21 (Figure 1 and SI).

The relative configuration of **1** was deduced by the NOESY experiment (Figure 1). The *syn* relative configuration of H-2 and H-22 could be assigned by NOE correlations between H-2 (δ_H 3.88) and H-22b (δ_H 1.89) and between H-2 and NH-28 (δ_H 8.59). The correlations between H-34 (δ_H 1.16) and H-22b indicated the *syn* orientation of H-23 (δ_H 3.22) and H-26 (δ_H 3.66). Diagnostic NOE correlations of axial H-21b (δ_H 1.53), Me-29 (δ_H 0.78), and Me-31 (δ_H 0.84) positioned these groups on the same side and *cis* with H-2. On the other hand, NOE correlations for H-22a/H-21a/H-5 and H-21a/H-20/H-14/H-30 indicated that these protons are on the other side of the drimane skeleton. The observed NOE correlations corroborated well the interatomic distances obtained from the computed low-energy conformer (87.5% population, Figure 2, *vide infra*) of indotertine A (**1**). Thus the relative configuration of **1** was deduced as (2*R**,3*S**,14*S**,19*S**,20*S**,23*S**,26*S**). For determination of the absolute configurations, the solution conformers and ECD spectra of **1** were calculated and compared with the experimental solution ECD spectrum (Figure 2). The solution TDDFT ECD calculation protocol has been found to be a powerful and reliable method for determining the absolute configuration of complex natural products,[8] and ECD spectra of indole alkaloids were also frequently correlated with their absolute configurations.[9] Above 225 nm, the experimental ECD spectrum of **1** is governed by the ^1L_b and ^1L_a transitions of the indole chromophore showing positive Cotton effects (CE) at 292 and 243 nm, respectively. The two high-energy ECD bands at 212 and 198 nm correspond to the ^1B transitions of indole, but they are overlapping with a number of other transitions as indicated by the ECD calculations as well. The MMFF conformational search of **1** followed by DFT optimization at the B3LYP/6-31G(d) level afforded three slightly different conformers above 0.5% population with 87.5%, 7.0%, and 5.0% populations, differring mainly in the orientation of the isopropyl group. Then ECD spectra of each major conformer were calculated with various functionals (B3LYP, BH&HLYP, PBE0) and the TZVP basis set. The Boltzmann-weighted ECD spectra of the (2*R*,3*S*,14*S*,19*S*,20*S*,23*S*,26*S*)-enantiomer reproduced well the experimental ECD curve with PBE0/TZVP giving the best agreement (Figure 2). Thus the absolute configuration of **1** was unambiguously determined as (2*R*,3*S*,14*S*,19*S*,20*S*,23*S*,26*S*). Since the two positive low-energy CEs of **1** derive from the ^1L_b and ^1L_a transitions of the indole chromophore, their signs are governed by the absolute configuration of C-2 and C-3, which in turn determines the *M* helicity ($\omega_{C\text{-}9,N\text{-}1,C\text{-}2,C\text{-}3} = -30.6°$ from conformer A) of the fused heteroring. Similarly to the

(7) Indotertine A (**1**): colorless amorphous solid; $[\alpha]^{20}_D$ +21.8 (*c* 0.15, MeOH); ECD (MeOH) λ [nm] ($\Delta\varepsilon$): 292 (+0.7), 243 (+5.6), 212 (+4.4), 198 (−8.5); IR (KBr) νmax: 3423, 2925, 1668, 1607, 1461, 1403, 1300 cm^{-1};UV (MeOH) λ_{max} (log ε): 208 (1.41), 202 (1.11); ^{1}H and ^{13}C NMR data, see Table S4 (Supporting Information); HRESIMS m/z: 504.3583 $[M + H]^+$ (calcd for $C_{32}H_{46}N_3O_2$, 504.3585).

(8) (a) Li, D. H.; Chen, L.; Zhu, T. J.; Kurtán., T.; Mándi, A.; Li, J.; Gu, Q. Q. *Tetrahedron* **2011**, *67*, 7913–7918. (b) Cai, Y. S.; Kurtán., T.; Miao, Z. H.; Mándi, A.; Komáromi, I.; Liu, H. L.; Ding, J.; Guo, Y. W. *J. Org. Chem.* **2011**, *76*, 1821–1830. (c) Hou, X. F.; Yao, S.; Mándi, A.; Kurtán., T.; Tang, C. P.; Ke, C. Q.; Li, X. Q.; Ye, Y. *Org. Lett.* **2012**, *14*, 460–463.

(9) (a) Parish, C. A.; Dong, J. G.; Bornmann, W. G.; Chang, J.; Nakanishi, k.; Berova, N. *Tetrahedron* **1998**, *54*, 15739–15758. (b) Tóth., G.; Clauder, O.; Gesztes, K.; Yemul, S. S.; Snatzke, G. *J. Chem. Soc., Perkin Trans. 2* **1980**, 701–703. (c) Koyama, K.; Hirasawa, Y.; Nugroho, A. E.; Kaneda, T.; Hoe, T. C.; Chan, K. L.; Morita, H. *Tetrahedron* **2012**, *68*, 1502–1506.

helicity rule of 2,3-disubstituted 2,3-dihydrobenzo[*b*]furan derivatives,[10] the correlation of the characteristic ECD transitions with the helicity of the indole heteroring may allow the configurational assignment of analogue indole derivatives simply from their ECD spectra.

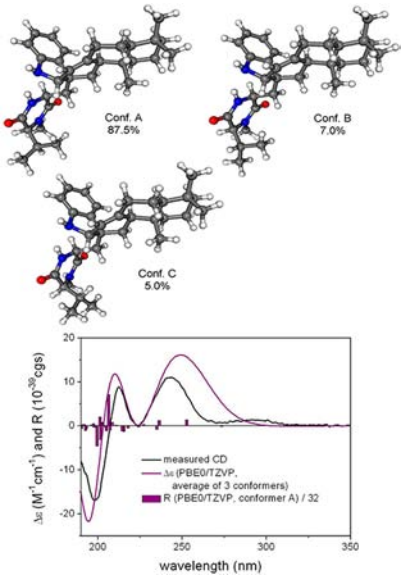

Figure 2. Low-energy B3LYP/6-31G(d) optimized conformers and populations of (2*R*,3*S*,14*S*,19*S*,20*S*,23*S*,26*S*)-**1** (top) and experimental ECD spectrum of indotertine A (**1**) compared with the PBE0/TZVP calculated ECD spectrum of the (2*R*,3*S*,14*S*, 19*S*,20*S*,23*S*,26*S*)- enantiomer (bottom).

Drimentine F (**2**)[11] was found to have a molecular formula of $C_{32}H_{45}N_3O_2$ based on the HRESIMS ion at *m*/*z* 504.3590 $[M + H]^+$, indicating that it possesses 12 degrees of unsaturation. ^1H and ^{13}C NMR spectral analysis (SI, Table S4) revealed that **2** is an isomer of drimentine A.[12] The main differences were attributed to the diketopiperazine ring, in which the leucine of drimentine A was replaced by a N-Me-valine residue, which was confirmed by the COSY correlations of H-3/H-25/H-26 (H-27) and HMBC correlations from H-26 (δ_H 1.22)and H-27 (δ_H 0.93) to C-3 (δ_C 67.7) and from H-28 (δ_H 2.91) to C-1 (δ_C 166.2) and C-3 (Figure 2).

The planar structure and absolute configuration of **2** has been confirmed by a single crystal X-ray diffraction analysis (Figure 3). The final refinement on the Cu Kα data resulted in a Flack parameter of 0.04, allowing an unambiguous assignment of the absolute configurations as (3*S*,5a*S*,10b*S*,11a*S*,13*S*,16a*S*,20a*S*). While the indole α and *β* carbons have opposite stereochemistry in **1** and **2**, which is also manifested in their opposite indole ^1L_b and ^1L_a CEs, the corresponding other chirality centers, C-14/C-16a, C-19/C-20a, C-20/C-13, C-23/C-11a, and C-26/C-3, are homochiral.

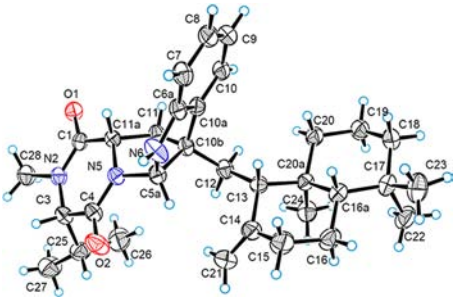

Figure 3. X-ray crystal structure of **2** (Cu Kα radiation).

Drimentine G (**3**)[13] was assigned the molecular formula $C_{31}H_{43}N_3O_2$ on the basis of an HRESIMS (*m*/*z* 490.3446 $[M + H]^+$) analysis, making it smaller that **2** by CH_2. The ^1H NMR data of **3** were largely identical to those of **2**. In fact, the only major difference was the one-proton singlet at δ_H 6.02 found in **3**, which suggested that the N−CH_3 of **2** was replaced by an NH as confirmed by the HMBC correlation from NH-2 to C-4 (δ_C 163.8) and C-11a (δ_C 58.3) (Figure 2).

The experimental ECD spectra of **2** and **3** were found to be very similar. CEs were observed at 196 nm (Δε −19.8) and 245 nm (Δε −4.0) for **3** and 206 nm (Δε −27.4) and 241 nm (Δε −5.3) for **2**, indicating the same absolute configurations of **2** and **3**.

Drimentines belong to a novel class of antibiotics possessing a new terpenylated diketopiperazine structure, which proved to exhibit antibiotic, antifungal, anticancer, and anthelmintic activities.[12] Up to now, the five reported drimentines (drimentines A−E), containing proline or leucine residues, were reported in a single patent, in which the determinations of the absolute configurations of drimentines were not clearly described,[6] and they or their derivatives have not been published in peer-reviewed journals. In our study, two new representatives of the drimentine family were identified, named drimentine F

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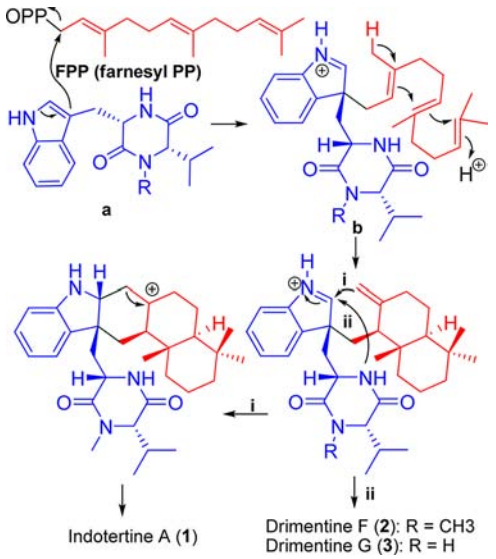
(11) Drimentine F (**2**): colorless amorphous solid; $[\alpha]^{20}_D$ −135.2 (*c* 0.10, MeOH); ECD (MeOH) λ [nm] (Δε) 297 (−2.2), 241 (−5.3), 206 (−27.4); IR (KBr) ν_{max} 3367, 2925, 1670, 1606, 1487, 1454, 1303 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (1.68), 243 (1.27); ^1H and ^{13}C NMR data, see Table S4 (SI). HRESIMS *m*/*z* 504.3590 $[M + H]^+$ (calcd for $C_{32}H_{46}N_3O_2$, 504.3585). To check the possibility for **1** to isomerize to **2**, perhaps through acid catalyzed ring opening followed by recyclization, we stirred **1** in methanol with 0.2% TFA for 24 h at RT and used TLC for detection. The result indicated that **1** is stable and does not transform to **2** at the condition we used.

(12) Lacey, E.; Power, M.; Wu, Z.; Rickards, R. W. Patent Int. Appl. 1998, WO 9809968 A1, 19980312.

(13) Drimentine G (**3**): colorless amorphous solid; $[\alpha]^{20}_D$ −86.1 (*c* 0.10, MeOH); ECD (MeOH) λ [nm] (Δε): 299 (−1.0), 270 (+0.5), 245 (−4.0), 220 (+2.6), 196 (−19.8); IR (KBr) ν_{max} 3448, 1668, 1460, 1112 cm^{-1}; UV (MeOH) λ_{max} (log ε) 209 (1.69), 243 (1.25); ^1H and ^{13}C NMR data, see Table S4 (SI). HRESIMS *m*/*z* 490.3446 $[M + H]^+$ (calcd for $C_{31}H_{44}N_3O_2$, 490.3434).

(**2**) and drimentine G (**3**), which, in contrast to the known derivatives, had valine residues. A patent application for drimentine G (**3**) had been filed for in China detailing the new structure and its cytotoxicities against HCT-8, Bel-7402, A549, and A2780 cell lines.[6]

Scheme 1. Plausible Biogenetic Pathway of Indotertine A (**1**) and Drimentines F (**2**) and G (**3**)



A plausible biogenetic pathway of indotertine A (**1**) and drimentines F (**2**) and G (**3**) is postulated in Scheme 1. The presumed intermediate **b** is first generated by the condensation of an amino acid derived precursor **a** and an isoprenoid precursor FPP which derived from the classical mevalonic acid pathway. Subsequently, a nucleophilic adddition to the α-position of the indole part could take place from either the terminal double bond (i) or amidic nitrogen (ii) to afford the pentacyclic product indotertine A (**1**) or tetracyclic product drimentines F (**2**) and G (**3**), respectively.[14]

(14) (a) Uchida, R.; Tomoda, H. *J. Antibiot*. **2006**, *59*, 298–302. (b) Ding, L.; Maier, A.; Fiebig, H. H.; Lin, W. H.; Hertweck, C. *Org. Biomol. Chem*. **2011**, *9*, 4029–4031. (c) Raju, R.; Piggott, A. M.; Conte, M.; Aalbersberg, W. G. L.; Feussner, K.; Capon, R. J. *Org. Lett*. **2009**, *11*, 3862–3865.

Compounds **1**–**3** were evaluated *in vitro* for their cytotoxicities against five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780 cells) using the MTT method[15] with taxol as a positive control (IC_{50}'s: 51, 6, < 1, 16, and < 1 nM, respectively). Among these, **3** showed the best cytotoxic activities against HCT-8, Bel-7402, A549, and A2780 cell lines, with IC_{50} values of 2.81, 1.38, 1.01, and 2.54 μM, respectively, while other compounds showed no significant cytotoxicities against the tested cell lines (IC_{50} > 10 μM) (Table S1, SI).

The molecule targets of **3** were also investigated in various models including topoisomerase I, topoisomerase II, and Hsp90. Only weak inhibitory activity was observed against topoisomerase I as assayed by relaxation of supercoiled plasmid DNA at 100 μM (Figure S1, SI).

In conclusion, three hybrid NRPS-terpenoid metabolites composed of sesquiterpenoid and amino acid components were isolated from a marine-derived actinomycetes CHQ-64. Indotertine A (**1**) represents a new condensed pentacyclic skeleton, in which the indole ring is fused with a sesquiterpene moiety. The co-occurrence of these HIs in a single strain suggested their biogenetic relationship, and thus a plausible biosynthetic pathway was proposed.

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Supporting Information Available. Detailed isolation procedure, spectrospic data, and details of the quantum chemical ECD calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

(15) Mosmann, T. J. *Immunol. Methods* **1983**, *65*, 55–63.

The authors declare no competing financial interest.