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Isoindolinone-type alkaloids from the sponge-derived fungus *Stachybotrys chartarum*

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ABSTRACT

Eight new isoindolinone-type alkaloids named chartarutines A–H (**1**–**8**) and a known analogue (**9**) were isolated from the sponge-associated fungus *Stachybotrys chartarum*. Their structures were elucidated by extensive spectroscopic (IR, MS, 1D, and 2D NMR) data analyses, in addition to the Mosher method and CD effects for the assignment of their absolute configurations. All compounds were evaluated for the inhibition against HIV-1 virus, while chartarutines B, G, and H exhibited significant inhibitory effects. Primary structure–activity relationship was discussed.

1. Introduction

Stachybotrin-type alkaloids are a small group of unusual natural products structurally characterized by the presence of a pyrano-isoindolinone nucleus bonded by a diisoprenyl group.[1,2] In biogenetic depiction, these meroterpenoid-based alkaloids are derived from farnesyl-bearing isoindolinone as a precursor, which is also suggested to be a precursor to derive spirodihydrobenzo furanlactams[3–9] through several steps of enzymatic cyclization. The antiviral and neuritogenic properties of isoindolinones attracted the interesting for chemists to discover diverse derivatives with structural novelty and pharmacological potency. So far, only three diisoprenyl-linked pyrano-isoindolinones named stachybotrins A–C have been reported,[1,2] whereas a number of isoindolinones linked by a triisoprenyl group through ether bonds were found from *Stachybotrys* species.[10] Our previous investigation of sponge-associated fungus *Stachybotrys chartarum* resulted in the isolation of 16 new phenylspirodrimanes with anti-hyperlipidemic effects in HepG2 cells.[8] In the course of our search for additional new isoindolinone derivatives, a large scale of fermentation with *S. chartarum* was performed. The HPLC/MS and NMR guided fractionation and purification of the EtOAc extract resulted in the isolation of eight new isoindolinone-based derivatives (**1**–**8**) together with a known analogue (Fig. 1).

2. Result and discussion

Based on the HPLC/ESIMS data for the identification of nitrogen-containing fingerprint peaks in association with the ^{1}H NMR data for the discrimination of the meroterpenoid features, the EtOAc extract of the fungus *S. chartarum* was separated by extensive column chromatography including semipreparative HPLC purification to obtain compounds **1**–**9**.

Chartarutine A (**1**) has a molecular formula of $C_{23}H_{33}NO_5$ as determined by the HRESIMS (*m*/*z* 426.2233 $[M+Na]^+$) and NMR data, requiring 8° of unsaturation. The ^{1}H NMR spectrum displayed an aromatic singlet at δ_H 6.63 (1H, s, H-6) an two olefinic triplets at δ_H 5.19 (1H, t, *J*=6.0 Hz, H-2′) and 5.09 (1H, *J*=6.0 Hz, H-6′), in addition to four methyl singlets and 13 alkyl protons. The ^{13}C NMR and DEPT spectra assigned a total of 23 carbon resonances, including six aromatic carbons, four olefinic carbons for two double bonds, a carbonyl carbon (δ_C 170.9, C-7), and two oxygen-bearing sp^3 carbons. The NMR data of **1** resembled those of stachybotrin B,[1] suggesting **1** to be structurally related to the known compound. An isoindolinone nucleus was recognized from the COSY coupling of NH (δ_H 8.26, br s) with the methylene protons at δ_H 4.13 (2H, br, H_2-8) in association with the HMBC

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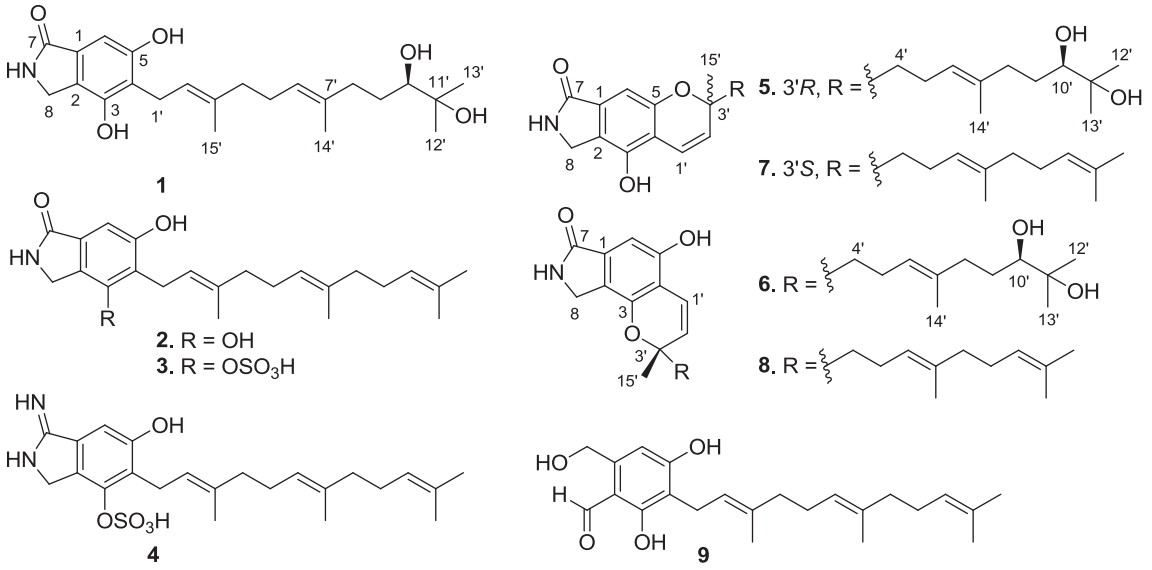

Fig. 1. Structures of the compounds isolated from *S. chartarum*.

interactions from NH and H_2-8 to the carbonyl carbon C-7, aromatic carbons at δ_C 131.7 (C-1) and 121.5 (C-2) and from the aromatic proton H-6 to C-1, C-2, C-4 (δ_C 119.1), and C-5 (δ_C 156.6). Additional HMBC interactions from the phenol proton of OH-5 (δ_H 9.50, s) to C-4, C-5, and C-6 (δ_C 100.7) and of OH-3 (δ_H 9.18, s) to C-2, C-3 (δ_C 150.8) and C-4 confirmed the substitution of phenol groups at C-3 and C-5, whereas a side chain was linked to C-4. The COSY and HMBC relationships established a linear side chain as 3′,7′,11′-trimethyldodeca-2′,6′-dien-10′,11′-diol. This moiety was linked to C-4 through C-1′/C-4 bond according to the HMBC interactions of H_2-1′ (δ_H 3.28, d, J=6.0 Hz) with C-3, C-4, and C-5 (Fig. 2). The NOE interactions between H-2′ (δ_H 5.19)/H_2-4′ (δ_H 1.90) and H-6′ (δ_H 5.09)/H_2-8′ (δ_H 1.80, 2.10) in combination with the methyl chemical shifts of C-14′ (δ_C 16.4) and C-15′ (δ_C 16.5) less than δ_C 20 ppm[11] deduced 2′*E* and 6′*E* geometries. The absolute configuration at C-10′ was assigned using the in situ dimolybdenum CD method.[12,13] adding dimolybdenum tetraacetate [$Mo_2(OAc)_4$] to a DMSO solution of **1**, a metal complex was generated as an auxiliary chromophore. The induced Cotton effects (ICE) reflected the O–C–C–O torsion angle of the vicinal diol. The negative ICE observed at 300 nm (Fig. 3) for the negative chirality indicated 10′*R* configuration.

The molecular formula of chartarutine B (**2**) was established as $C_{23}H_{31}NO_3$ according to the HRESIMS (m/z 392.2180 $[M+Na]^+$) and NMR data. Comparison of the NMR data (Tables 1 and 2) revealed that the 1,2-diol resonances of **1** were replaced by a double bond resonated at δ_C 124.6 (C-10′) and 131.0 (C-11′) in **2**, whereas the remaining NMR data of both **1** and **2** were very similar. The COSY and HMBC correlations of **2** further supported the side chain as a 3′,7′,11′-trimethyldodeca-2′,6′, 10′-triene. Thus, the structure of **2** was determined as 10′,11′-dehydroxy-10′,11′-ene analogue of **1**, while the absolute configurations of **2** were established to be the same as those of **1** by the results of X-ray diffraction (Fig. 4).

The NMR data of chartarutine C (**3**) were virtually similar to those of **2**, except for its molecular formula ($C_{23}H_{31}NO_6S$) presenting a SO_3 unit more than that of **2** as determined by the HRESIMS (m/z 448.1804 $[M-H]^-$) data. The daughter ion at m/z 369 $[M-SO_3H]^+$ produced by the EIMS fragmentation supported the sulfate assignment. This finding in association with the upfield shift of C-3 (δ_C 147.3) and downfield shifts of C-2 (δ_C 129.0) and C-4 (δ_C 129.0) suggested the sulfate unit to be substituted at C-3.

The molecular formula ($C_{23}H_{32}N_2O_5S$) of chartarutine D (**4**) differed from that of **3** due to the presence of an additional NH group and absence of an oxygen atom. Comparison of the NMR data

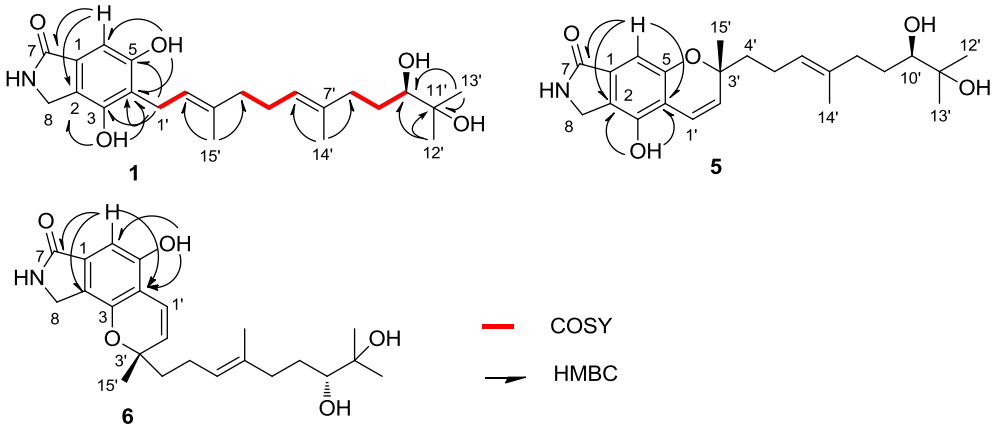

Fig. 2. Key COSY and HMBC correlations of **1**, **5**, and **6**.