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Integracides F and G: New tetracyclic triterpenoids from the endophytic fungus *Fusarium* sp.

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ABSTRACT

Two new tetracyclic triterpenoids: integracides F (**1**) and G (**2**) have been isolated from the endophytic fungus *Fusarium* sp. isolated from the roots of *Mentha longifolia* L. (Labiatae) growing in Saudi Arabia. Their structures were established by UV, IR, 1D (^{1}H and ^{13}C), 2D (^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY) NMR, and HRESIMS spectral data, in addition to comparison with literature data. The isolated compounds were evaluated for their anti-microbial, anti-malarial, anti-leishmanial, and cytotoxic activities. Compound **1** and **2** displayed potent cytotoxic activity towards BT-549 and SKOV-3 with IC_{50} values of 1.97 and 0.16 μg/mL and 1.76 and 0.12 μg/mL, respectively compared to doxorubicin (IC_{50} 1.61 and 0.095 μg/mL, respectively). Moreover, they exhibited significant anti-leishmanial activity towards *Leishmania donovani* with IC_{50} values of 3.74 and 2.53 μg/mL, respectively and IC_{90} values of 5.11 and 8.89 μg/mL, respectively.

1. Introduction

Endophytic microorganisms are bacteria or fungi that live inside plant tissues, without causing damage or disease symptoms to their hosts (Elkhayat et al., 2015; deSouza et al., 2011). The secondary metabolites produced by these microorganisms are a valuable repository of natural bioactive compounds, many of which have been identified as useful research reagents and potential drug candidates (Ibrahim et al., 2015; Meinwald and Eisner, 2008; Geris dos Santos and Rodrigues-Fo, 2003). *Fusarium* sp. are a widespread cosmopolitan group of fungi and commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders (El-Kazzaz et al., 2008). *Fusarium* sp. is well known for the production of integracides, which are a class of a tetracyclic 4,4-dimethylergostane triterpenoids containing a 12-acetyl-$\Delta^{8,14}$-diene-11-ol moiety. They have been shown to possess elastase, rhinovirus 3C protease, HIV-1 integrase, and cholesteryl ester transfer protein inhibitory activities (Singh et al., 2003a,b; Singh, 2000; Tabata et al., 1999; Brill et al., 1996). As part of an ongoing search for bioactive compounds from endophytic fungi, we have identified two new tetracyclic triterpenoids: integracides F (**1**) and G (**2**) from *Fusarium* sp. isolated from the roots of *Mentha longifolia* L. (Fig. 1). The fungal EtOAc extract was subjected to Sephadex LH-20, silica gel, and RP-18 column chromatography to yield compounds **1** and **2**. Herein, we report the isolation and structure elucidation as well as anti-microbial, anti-malarial, anti-leishmanial, and cytotoxic activities of the new compounds.

2. Results and discussion

Compound **1** was obtained as colorless powder. Its HRESIMS spectrum gave a pseudo-molecular ion peak at *m*/*z* 557.3839 (calcd. for $C_{34}H_{53}O_6$, 557.3842 $[M+H]^+$) compatible with the molecular formula $C_{34}H_{52}O_6$, requiring nine degrees of unsaturation. The IR spectrum showed characteristic absorption bands at 3435 (hydroxyl group), 1723 (ester group), and 1664 and 885 (exocyclic *di*-substituted double bond) cm^{-1}. The UV spectrum showed an absorption band at λ_{max} 248 nm characteristic for a heteroannular diene system (Singh et al., 2003b). Compound **1** was 43 mass units and one degree of unsaturation more than intergracide B, indicating the presence of an additional acetyl group in **1**. The NMR spectral data of **1** were similar to intergracide B previously isolated from *Fusarium* sp. (Singh et al., 2003a,b). The

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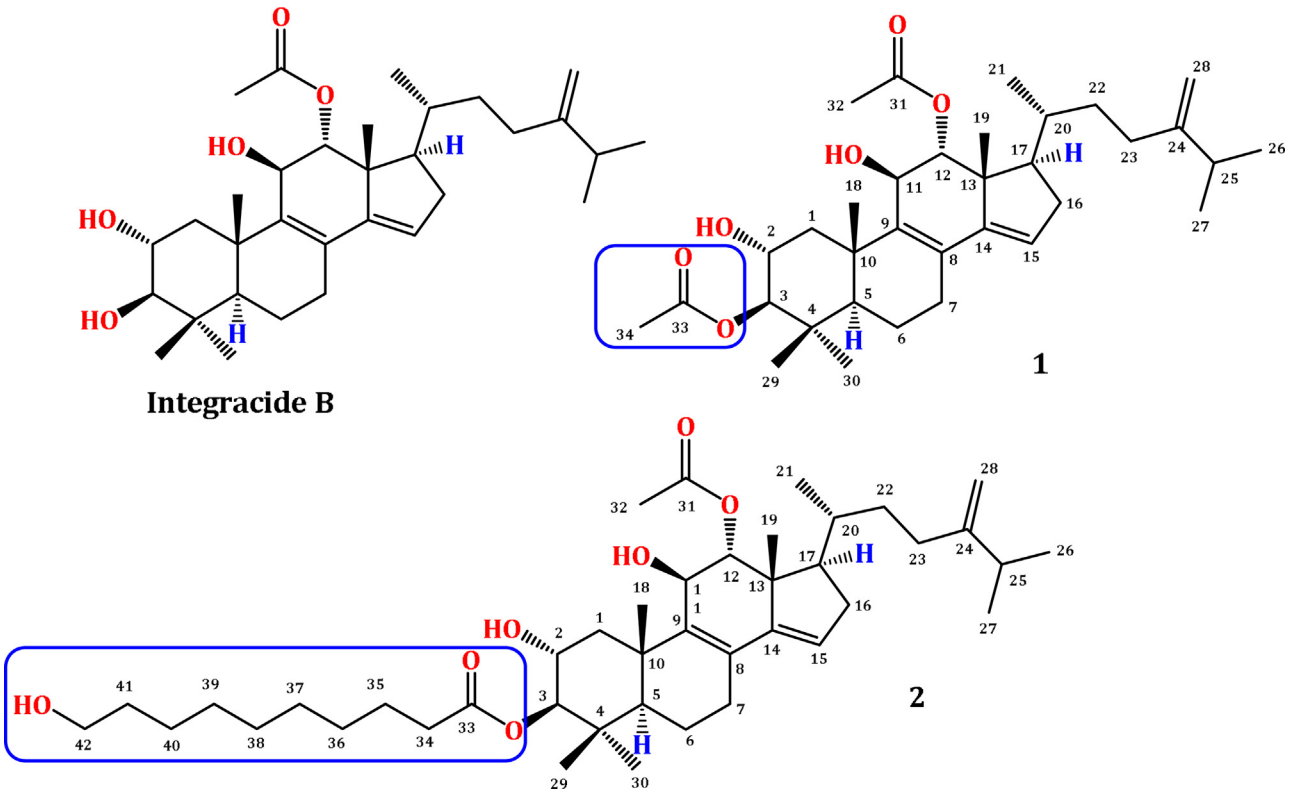

Fig. 1. Structures of integracides B, F (**1**), and G (**2**).

^{13}C and HSQC NMR spectra of **1** displayed resonances for 34 carbon signals: 9 methyls, 7 methylenes, 9 methines four of them for oxymethine carbons, and 9 quaternary carbons, including 2 carbonyls and three olefinic carbons. In the ^{1}H and ^{13}C NMR spectra, signals for a tri-substituted olefinic double bond were observed at δ_H 5.52 (brt, J = 2.5 Hz, H-15)/δ_C 120.7 (C-15) and 147.5 (C-14) (Table 1). It was positioned at C_{14}-C_{15} based on the HMBC correlations of H-15 to C-8, C-16, and C-17 and H-12, H-16, and H-19 to C-14 and the ^{1}H-^{1}H COSY cross peaks (Figs. 2 and 3) of H-15 to the methylene protons at δ_H 2.39 (m, H-16A) and 2.00 (m, H-16B). Furthermore, the ^{1}H and ^{13}C NMR showed signals at δ_H 4.71 (brs, H-28A) and 4.66 (brs, H-28B)/δ_C 107.0 (C-28) and 156.2 (C-24), indicating the presence of an exomethylene group. Its position was established by the ^{3}J HMBC cross peaks of H-28 to C-23 and C-25, H-22, H-26, and H-27 to C-24, and H-23 and H-25 to C-28. Moreover, four singlet methyl groups at δ_H 1.21 (H-18)/δ_C 23.1 (C-18), 0.98 (C-19)/17.0 (C-19), 0.78 (H-29)/17.9 (C-29), and 0.96 (H-30)/29.1 (C-30) and doublet methyl group at δ_H 0.86 (d, J = 6.2 Hz, H-21)/δ_C 18.3 (C-21) were observed. The HMBC spectrum (Fig. 3) showed cross peaks from H-18 to C-1, C-5, C-9, and C-10, H-19 to C-12, C-13, C-14, and C-17, H-29 and H-30 to C-3, C-4, and C-5, and H-21 to C-17, C-20, and C-22, establishing the locations of the methyl groups at C-10, C-13, C-4, and C-20, respectively. The presence of an isopropyl moiety in **1** was evident from the signals at δ_H 0.99 (3H, d, J = 6.5 Hz, H-26)/δ_C 22.2 (C-26), 1.00 (3H, d, J = 6.5 Hz, H-27)/δ_C 22.1 (C-27), and 2.19 (1H, m H-25)/33.5 (C-25) and confirmed by ^{1}H-^{1}H COSY correlations (Fig. 2) of H-26 and H-27 with H-25. The connectivity of isopropyl moiety at C-24 was confirmed by the HMBC correlations of H-25 to C-23, C-24, and C-28. The signals at δ_H 3.76 (1H, ddd, J = 11.4, 10.1, 4.0 Hz, H-2)/δ_C 67.3 (C-2), 3.59 (1H, d, J = 10.1 Hz, H-3)/88.5 (C-3), 4.09 (1H, brs, H-11)/67.9 (C-11), and 4.97 (1H, d, J = 2.1 Hz, H-12)/78.2 (C-12) indicated the presence of four oxygen-bonded methine groups. They were positioned at C-2, C-3, C-11, and C-12, respectively based on the observed ^{1}H-^{1}H COSY and HMBC correlations of H-1 to C-2 and C-3, H-5, H-29, and H-30 to C-3, H-12 to C-11, and H-17 and H-19 to C-12 (Fig. 3). The ^{13}C NMR spectrum displayed signals at δ_C 124.2 and 139.9 characteristic for the presence of a tetra-substituted olefinic double bond. Its placement at C_8-C_9 was secured by the HMBC cross peaks of H-6, H-11, and H-15 to C-8 and H-7, H-12, and H-18 to C-9. The ^{1}H NMR spectrum showed two singlet methyl signals at δ_H 2.05 (H-32) and 2.01 (H-34), correlating to the carbon signal resonating at δ_C 21.4 (C-32, 34) in the HSQC spectrum. Also, they showed HMBC cross peaks to the carbonyl carbons at δ_C 170.5 (C-31) and 170.3 (C-33), respectively, indicating the presence of two acetoxy moieties in **1**. This was confirmed by the ESIMS fragment ion peaks at m/z 514 $[M + H\text{-}COCH_3]^+$ and 471 $[M + H\text{-}2 \times COCH_3]^+$. The HMBC cross peaks (Fig. 3) of H-3 to C-33 and H-12 to C-31 established the connectivity of the acetoxy groups at C-3 and C-12, respectively. The ^{1}HNMR spectrum of **1** showed two singlet signals at δ_H 5.32 and 5.38 which were assigned to 2-OH and 11-OH groups, respectively (Table 1). Their assignment was secured by the HMBC cross peaks of 2-OH to C-1, C-2, and C-3 and 11-OH to C-9, C-11, and C-12. The relative configuration of **1** was assigned based on the comparison of the ^{1}H and ^{13}C chemical shifts as well as coupling constant values of **1** with literature and further confirmed by the NOESY experiment (Singh et al., 2003a,b). The NOESY spectrum showed correlations of H-3 to H-5, H-11 and H-17 to H-5, and H-11 to H-21, indicating that these protons occurred on the same side of the molecule. Moreover, the NOESY cross peaks of H-2 to H-12 and H-18 and H-12 to H-20 positioned these protons on the other side of the molecule (Fig. 2). On the basis of these evidences and by comparison of NMR data of **1** with those of the previously reported integracides, the structure of **1** was unambiguously elucidated and named integracide F.

Compound **2** was isolated as white amorphous powder and its molecular formula was determined as $C_{42}H_{68}O_7$ by the HRESIMS