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Sesquiterpene coumarins from *Ferula szowitsiana* and *in vitro* antileishmanial activity of 7-prenyloxycoumarins against promastigotes

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Abstract

Two new sesquiterpene coumarins, named szowitsiacoumarin A (**1**) and szowitsiacoumarin B (**2**), and a phenylpropanoid derivative, 2-epihelmanticine (**3**), together with nine known compounds, auraptene (**4**), umbelliprenin (**5**), galbanic acid (**6**), methyl galbanate (**7**), farnesiferol B (**8**), farnesiferol C (**9**), persicasulfide A (**10**), β-sitosterol and stigmasterol were isolated from the roots of *Ferula szowitsiana*. The structures of these compounds were elucidated by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, and ROESY) as well as HR-MALDI-MS analysis. Since the configuration of 2-epihelmanticine was previously only partly determined, a relative configurational analysis of its four stereocenters was carried out on the basis of the recently reported *J*-based method.

The inhibiting activity of prenylated coumarins, auraptene (**4**) and umbelliprenin (**5**), in addition to galbanic acid (**6**), as major component, and of the Me_2CO extract of *Ferula szowitsiana* (Apiaceae) roots has been evaluated against promastigotes of *Leishmania major*. Umbelliprenin and auraptene showed significant activity with IC_{50} values of 4.9 μg/ml (13.3 μM) and 5.1 μg/ml (17.1 μM) after 48 h incubation, respectively.

Keywords: *Ferula szowitsiana*; Szowitsiacoumarin A; Szowitsiacoumarin B; 2-Epihelmanticine; Auraptene; Galbanic acid; Umbelliprenin; *Leishmania major*; Promastigotes

1. Introduction

Leishmaniasis is a parasitic disease, in which the sand fly is the common vector of transmission. Species of the genus *Leishmania*, a protozoan member of the hemoflagelate group, are causative agents of human leishmaniasis, which has a reservoir in rodents, dogs and others in the wild animal population, and transmitted by mosquitoes of the genera *Lutzomia* and *Phlebotomus*. Members of the genus *Leishmania* differentiate from proliferative promastigotes in the sand fly vector gut to infective metacyclic promastigotes in the insect foregut. Parasites are inoculated by the vector as the flagellate promastigotes enter the mammalian host, where they infect macrophages, differentiating into nonmotile amastigotes and multiply as such (Carvalho et al., 2000). The term leishmaniasis comprises three clearly distinguishable clinical manifestations: generalized visceral infection, cutaneous leishmaniasis, and mucocutaneous leishmaniasis.

Leishmaniasis is regarded as a major public problem (WHO), causing significant morbidity and mortality in Africa, Asia and Latin America. With an estimated number of 500,000 new cases accruing annually, visceral

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leishmaniasis is still considered as one of the most severe affections by the World Health Organization (WHO. Fact sheet No 116).

The treatment of leishmaniasis is difficult because of the intramacrophagic location of the infectious form. In the absence of a vaccine, there is an urgent need for effective drugs to replace or supplement those in current use. The clinically used drugs, many of which are based on pentavalent antimony compounds, were developed before 1959. The toxicity of these agents and the persistence of side-effects even after modification of dose level and duration of treatment are, however, severe drawbacks. The search for antileishmanial agents has been exhaustive. Alternative drugs, such as amphotericin B and pentamidine, also have unpleasant side effects (Balana et al., 1998; Carvalho et al., 2000). On the other hand, plant extract or plant-derived compounds are likely to provide a valuable source of new medicinal agents (Kayser et al., 2001; Carvalho and Ferreira, 2001).

Recently, many natural products have been reported to show antileishmanial activity including naphthoquinones, lignans, neolignans, alkaloids, chalcones and triterpenoids (Kayser et al., 2000; Sauvain et al., 1996; Barata et al., 2000; Delorenzi et al., 2001; Torres-Santos et al., 1999; Camacho et al., 2000). Coumarins have been also reported to show antileishmanial activity (Oketch-Rabah et al., 1997; Bravo et al., 1999) and it was recently reported that auraptene can inhibit the growth of promastigotes of *Leishmania major* ($LD_{50} = 30\ \mu M$) (Napolitano et al., 2004).

The exclusively old world genus *Ferula* belongs to the family Umbelliferae with about 130 species distributed throughout the Mediterranean area and central Asia, specially in the former USSR and neighboring countries such as Iran. This genus is well documented as a good source of biologically active compounds such as sesquiterpene derivatives (Ahmed et al., 2001; Ahmed, 1999; Valle et al., 1987). Sesquiterpene derivatives, especially sesquiterpene coumarins, were stored in the roots of the plants; therefore the roots are better source for isolating sesquiterpene coumarins than the aerial parts. *Ferula szowitsiana*, similar to other species of the genus *Ferula*, is a rich source of sesquiterpene coumarins (Murray et al., 1982). In the present study, we report the isolation and the structure elucidation of two new compounds, szowitsiacoumarin A, szowitsiacoumarin B, (**1**–**2**) along with the isolation and the NMR configurational assignment of 2-epihelmanticine (**3**). We also isolated persicasulfide A (**10**) and the two common well-known steroids, β-sitosterol and stigmasterol, for the first time from *F. szowitsiana*, together with auraptene (**4**), umbelliprenin (**5**), galbanic acid (**6**), methyl galbanate (**7**), farnesiferol B (**8**), farnesiferol C (**9**) (Fig. 1). On the basis of the antileishmanial effect of auraptene, a prenylated coumarin, we tested the growth inhibitory activity of umbelliprenin (**5**), galbanic acid (**6**) and of the Me_2CO extract of the roots against *L. major* promastigotes.

2. Results and discussion

The isolated coumarins (**4**–**9**) (Murray et al., 1982; Lee et al., 1998; Iranshahi et al., 2003b; Nabiev et al., 1982), persicasulfide A (**10**) (Iranshahi et al., 2003a) and β-sitosterol and stigmasterol (Goad and Akihisa, 1997) were identified by comparison of their NMR, IR, MS and melting point data with those previously described in the literature. This is the first report of persicasulfide A, a sulfur containing compound, β-sitosterol and stigmasterol from *F. szowitsiana*. Other components including six coumarins (**4**–**9**) were previously reported from the plant (Murray et al., 1982).

Szowitsiacoumarin A (**1**) showed a pseudomolecular ion peak in the HR-MALDI-MS spectrum at *m*/*z* of 383.2195 $[M + H]^+$, consistent with the molecular formula $C_{24}H_{31}O_4$ (exact mass calculated for $C_{24}H_{31}O_4$ 383.2222). The structure of **1** was established from analysis of the ^1H and ^{13}C NMR spectra (Table 1). Compound **1** displayed 24 carbon signals, nine being typical of an umbelliferone skeleton and the other 15 signals were ascribable to a sesquiterpene moiety. The downfield signal at δ_C 161.2 was assigned to the carbonyl carbon of the coumarin moiety, whereas the downfield signal at δ_C 212.5 was indicative of a ketone group belonging to the sesquiterpene unit. HSQC spectrum classified the carbon signals to four aliphatic methylenes at δ_C 23.6, 25.4, 32.3, and 41.4, to a primary alcoholic carbon at δ_C 75.9 characteristic for C-11′, to nine methines, five of them for umbelliferone moiety at δ_C 113.0 (C-3), 143.3 (C-4), 128.7 (C-5), 113.1 (C-6), and 101.2 (C-8) and to four methyls at δ_C 7.1, 14.4, 14.9, and 20.0. The ^1H NMR showed for four three-proton signals two singlets in accordance with two tertiary methyl groups and two doublets in accordance with two secondary methyl groups.

Long range HMBC correlations from the proton signal at δ_H 1.13 (Me-12′) to the carbon resonance at δ_C 39.5 (C-9′) and from the protons of the primary oxygenated carbon C-11′ to the same carbon C-9′ revealed the location of a tertiary methyl group (Me-12′) at C-9′.

A third HMBC correlation between the doublet methyl (Me-15′) at δ_H 1.03 and the carbon resonance at δ_C 39.5 (C-9′) allowed to assign a first doublet methyl at C-8′. The remaining methyl groups (Me-13′ and Me-14′) were established at C-4′ and at C-5′ carbons, respectively, from the COSY cross-peak between the quartet (H-4′) at δ_H 2.36 and the doublet methyl (Me-13′) at δ_H 0.93 and from the HMBC correlation between the carbon resonance at δ_C 58.3 (C-4′) and the proton signals at δ_H 0.82 (Me-14′). The down-field carbon chemical shift at δ_C 58.3 was consistent with a neighboring group effect from a C-3′ ketone.

The ROESY experiment supported the relative configuration of the stereogenic centres at C-4′, C-5′, C-8′, C-9′, and C-10′.

In particular ROEs H-11′/Me-15′, H-10′/Me-15′ established an α-orientation for both H-10′ and Me-15′, and a β-orientation for Me-12′. On the other hand, H-10′ showed correlation with H-4′, whereas Me-14′ with H-7′β at δ_H 1.97 which in turn exhibited correlation with Me-12′. These