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Microbial metabolism of cannflavin A and B isolated from *Cannabis sativa*

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ABSTRACT

Microbial metabolism of cannflavin A (**1**) and B (**2**), two biologically active flavonoids isolated from *Cannabis sativa* L., produced five metabolites (**3**–**7**). Incubation of **1** and **2** with *Mucor ramannianus* (ATCC 9628) and *Beauveria bassiana* (ATCC 13144), respectively, yielded 6″*S*,7″-dihydroxycannflavin A (**3**), 6″*S*,7″-dihydroxycannflavin A 7-sulfate (**4**) and 6″*S*,7″-dihydroxycannflavin A 4′-*O*-α-L-rhamnopyranoside (**5**), and cannflavin B 7-*O*-β-D-4‴-*O*-methylglucopyranoside (**6**) and cannflavin B 7-sulfate (**7**), respectively. All compounds were evaluated for antimicrobial and antiprotozoal activity.

1. Introduction

Cannabis sativa L. (Family: Cannabaceae), a plant originating in Central Asia, is cultivated worldwide as a source of fiber, energy, food and medicinal or narcotic preparations (Flores-Sanchez and Verpoorte, 2008). Recently, a number of new cannabis constituents were identified, increasing the total from 489 in 2005 (ElSohly and Slade, 2005) to 537 in 2009 (Ahmed et al., 2008a,b; Appendino et al., 2008; Radwan et al., 2009, 2008a,b), while the number of cannabinoids increased from 70 to 109. The cannabinoids are the most studied cannabis constituents, in particular Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive component (Galal et al., 2009); however, research has shown that some of the other cannabinoids also exhibit pharmacological activities, e.g., the nonpsychotropic cannabinoid cannabidiol (CBD) displays antihyperalgesic, antipsychotic, anticonvulsant, neuroprotective and antiemetic properties (Galal et al., 2009). Other classes of compounds reported for cannabis include terpenes, sugars, hydrocarbons, steroids, flavonoids, nitrogenous compounds, noncannabinoid phenols and amino acids (ElSohly and Slade, 2005).

More than 4000 flavonoids have been identified and numerous beneficial health effects have been reported, e.g., antiinflammatory (Gomes et al., 2008), antiviral (Naithani et al., 2008), anticancer (Mojzisova and Mojzis, 2008), cardioprotective (Mojzisova and Mojzis, 2008), antioxidant (Ibrahim et al., 2008), antiprotozoal (Fotie, 2008) and antimicrobial (Heinonen, 2007) activities.

Twenty-six flavonoids have been isolated from cannabis (ElSohly and Slade, 2005; Flores-Sanchez and Verpoorte, 2008; Radwan et al., 2008a), representing seven chemical structures (vitexin, isovitexin, apigenin, luteolin, kaempferol, orientin and quercetin) with different glycosylation, prenylation, geranylation and methylation patterns. Cannflavin A and B, two methylated isoprenoid flavones, represent the first aglycone flavonoids uniquely isolated from cannabis. The antileishmanial activity for cannflavin A and B was reported as strong (IC_{50} 10.3 μM) (Radwan et al., 2008a) and moderate (IC_{50} 13.6 μM) (Radwan et al., 2008b), respectively.

Microorganisms are used as predictive models for mammalian drug metabolism to establish the metabolic fate of biologically active compounds, while providing sufficient amounts of metabolite for structure elucidation and pharmacological evaluation.

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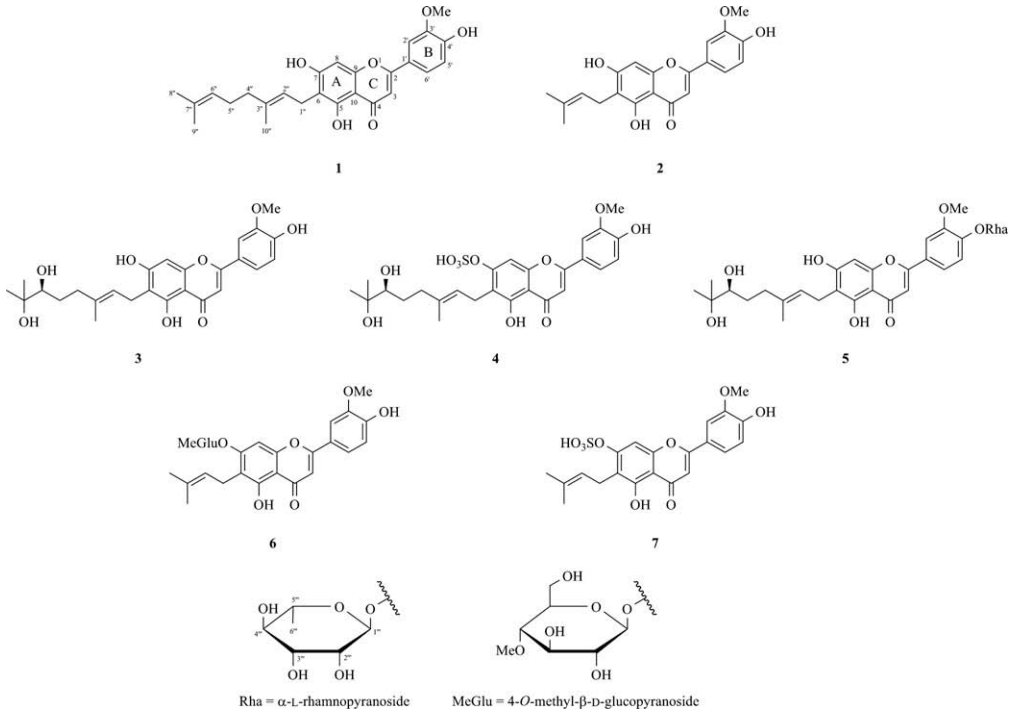
These models are also employed to obtain more active or less toxic substances and selective derivatives (Venisetty and Ciddi, 2003).

We herein report the microbial metabolism of **1** and **2** via a panel of 41 microorganisms, as well as the isolation, structure elucidation and activity (antimicrobial and antiprotozoal) of the resultant metabolites.



2. Results and discussion

Initial screening of **1** and **2** was carried out using a standard two-stage procedure (Ibrahim et al., 2008), with three and five of the 41 microorganisms showing the ability to transform **1** (three polar metabolites) and **2** (two polar metabolites), respectively. TLC analysis indicated that *Mucor ramannianus* (ATCC 9628) and *Beauveria bassiana* (ATCC 13144) had the highest transformational efficiencies for **1** and **2**, respectively, and was thus chosen for scale-up fermentation. Preparative scale fermentation of **1** with *M. ramannianus* yielded three new metabolites [6″*S*,7″-dihydroxycannflavin A (**3**), 6″*S*,7″-dihydroxycannflavin A 7-sulfate (**4**) and 6″*S*,7″-dihydroxycannflavin A 4′-*O*-α-L-rhamnopyranoside (**5**)], while preparative scale fermentation of **2** with *B. bassiana* yielded two new metabolites [cannflavin B 7-*O*-β-D-4‴-*O*-methylglucopyranoside (**6**) and cannflavin B 7-sulfate (**7**)].

Metabolite **3** was isolated as a light yellow powder with molecular formula $C_{26}H_{30}O_8$ based on HRESIMS and ^{13}C NMR spectroscopic data (12° of unsaturation). The UV λ_{max} at 275 and 350 nm indicated a flavonoid structure (Avula et al., 2009). The ^{13}C and DEPT-135 NMR spectroscopic data displayed an sp^3 oxymethine (δ_C 72.3) and an sp^3 geminal dimethyl oxygenated quaternary carbon (δ_C 77.7), in addition to eight oxygenated carbon resonances similar to those found in **1** (Choi et al., 2004). A comparison of the ^1H (Table 1) and ^{13}C (Table 2) NMR data of **1** and **3** indicated almost identical resonances for both compounds, except for the replacement of the C-6″/C-7″ olefinic resonances in **1** [δ_H 5.04 (*t*, *J* = 7.0, H-6″); δ_C 125.2 (C-6″), 131.6 (C-7″)] by oxymethine [δ_H 3.80 (m); δ_C 77.7 (C-6″)] and oxygenated quaternary carbon [δ_C 72.3 (C-7″)] resonances in **3**. A series of HMBC (H-6″/C-4″, C-8″, C-9″; C-6″/H_2-4″, H_3-8″, H_3-9″; C-7″/H_3-8″, H_3-9″; H_2-5″/C-9″) and COSY (H-6″/H_2-5″/H_2-4″) correlations confirmed the assignment of hydroxy groups at C-6″ and C-7″, establishing **3** as 6″,7″-dihydroxycannflavin A.

Metabolite **4**, a yellow amorphous powder, was shown to have a molecular formula $C_{26}H_{30}O_{11}S$ (HRESIMS). The IR spectroscopy displayed characteristic sulfate bands at ν_{max} 1050 (C–O–S) and 1250 (S=O) cm^{-1} (Ibrahim, 2005). The ^1H (Table 1) and ^{13}C (Table 2) NMR chemical shifts of **4** and **1** were similar, with significant differences being the downfield shift of H-8, C-6, C-8 and C-10 (0.55, 2.5, 4.0 and 0.6 ppm, respectively) and the upfield shift of C-7 (1.9 ppm), supporting the sulfation of the C-7 hydroxy (Barron and Ibrahim, 1987). The C-6 geranyl group in **4** was 6″,7″-dihydroxylated as in **3** based on HMBC (H-6″/C-4″, C-8″, C-9″; C-6″/H_3-8″; C-7″/H_3-8″, H_3-9″) and COSY (H-6″/H_2-5″/H_2-4″) correlations and the absence of C-6″/C-7″ olefinic NMR resonances, establishing **4** as 6″,7″-dihydroxycannflavin A 7-sulfate.

Metabolite **5** (HRESIMS: $C_{32}H_{40}O_{12}$), isolated as a light yellow powder, displayed similar ^1H (Table 1) and ^{13}C (Table 2) NMR resonances to **3** and **4**, with additional resonances indicating a pyranose moiety [^1H NMR: δ_H 5.50 (1H, *br s*, H-1‴), 1.04 (3H, *d*, *J* = 6.0, H_3-6‴); ^{13}C NMR: δ_C 98.4 (C-1‴), 16.1 (C-6‴)]. The pyranoside location was determined at C-4′ via HMBC correlation of the anomeric proton and C-4′ (δ_C 149.0), while its identity was determined through GC–MS analysis. The acetylated thiazolidine derivative of the hydrolysis product of **5** and of authentic L-rhamnose displayed the same GC–MS retention time (15.64 min) (Shukla et al., 2009). The chemical shift of the anomeric carbon [δ_C 98.4 (C-1‴)] suggested an α-*O*-glycosidic linkage (Herath et al., 2008; Ibrahim et al., 2008), establishing **5** as 6″,7″-dihydroxycannflavin A 4′-*O*-α-L-rhamnopyranoside.

The absolute configuration at C-6″ was determined through application of the modified Mosher's method ($\Delta\delta = \delta_S - \delta_R$) for **5** (Ahmed et al., 2008a; Murata et al., 2008; Nakamura et al., 2009; Zhang et al., 2009). The ^1H NMR resonances for the H_3-8″ and