Microbial Metabolism. Part 8.1) The Pyranocoumarin, Decursin

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Microbial transformation of the cancer chemopreventive agent, decursin (1) with Sepedonium chrysospermum (ATCC 13378) yielded two metabolites, (+)-decursinol (2) and (−)-cis-decursidinol (3). The structures were established by spectroscopic data.

Key words decursin; microbial metabolism; Sepedonium chrysospermum

The genus Angelica of the plant family Apiaceae includes over sixty species which are medicinally important. They are used to treat many illnesses including, colds, hepatitis, rheumatism, typhoid and fungal infections and many other disorders, apart from being utilized them as anti-inflammatory and diuretic agents. Angelica gigas is one such species whose roots have been used to treat anemia. It is also used as a sedative, under the Korean name Zam Dang Gui. Methanolic extract of the roots of the plant inhibits acetylcholinesterase enzyme (AChE) activity. AChE is responsible for the hydrolysis of acetylcholine (ACh), which is needed to increase the cholinergic functions in the brain. ACh deficiency leads to memory impairments in Alzheimer’s disease. In vitro, AChE activity guided isolation yielded several coumarins with considerable activity. Whilst the coumarin, decursinol (2), exhibited the highest AChE inhibitory activity, its structural analogue, decursin, showed relatively poor activity. Structure activity data suggested the need for a free hydroxyl group at C-3 for AChE activity. It is interesting however, to observe the two compounds exhibiting a reverse effect as anticancer agents on certain human prostate carcinoma cells indicating the importance of the substituted side chain in decursin for anticancer efficacy. The importance of the senecioic acid moiety of decursin as a coumarin moiety (3) due to the 3-methylbut-2-enoate side chain. The presence of –OH, –CH, –C=O groups, in the compound was suggested by the IR absorption bands at 3440, 2979, 1626 and 1717 cm⁻¹. Its ¹H- and ¹³C-NMR spectra differed from those of decursin (1) by the absence of signals due to the 3-methylbut-2-enolate side chain. The presence of a coumarin moiety (δ 6.20 and 7.57, a doublet of 1H each due to H-3 and H-4) and a pair of aromatic protons para to each other (δ 6.80 and 7.17, a singlet of 1H each), along with a geminal dimethyl group (δ 1.36 and 1.39, a singlet of 3H each) and a –CH₂–CH system (δ 2.83 and 3.11, 1H each and δ 3.86 due to H-3’ proton) indicated its similarity to decursinol. Comparison of the reported specific rotation and NMR data enabled to characterize the metabolite as (+)-decursinol (2).

Decursinol (2) (5 mg, 1.25% yield) was a white solid with a molecular formula C₁₄H₁₂O₅ (HR-ESI-MS data). The presence of –OH, –CH, –C=O groups, in the compound was suggested by the IR absorption bands at 3440, 2979, 1626 and 1717 cm⁻¹. Its ¹H- and ¹³C-NMR spectra differed from those of decursin (1) by the absence of signals due to the 3-methylbut-2-enolate side chain. The presence of a coumarin moiety (δ 6.20 and 7.57, a doublet of 1H each due to H-3 and H-4) and a pair of aromatic protons para to each other (δ 6.80 and 7.17, a singlet of 1H each), along with a geminal dimethyl group (δ 1.36 and 1.39, a singlet of 3H each) and a –CH₂–CH system (δ 2.83 and 3.11, 1H each and δ 3.86 due to H-3’ proton) indicated its similarity to decursinol. Comparison of the reported specific rotation and NMR data enabled to characterize the metabolite as (+)-decursinol (2).

Decursidinol (3) (5 mg, 1.25% yield) was isolated as a white solid. The HR-ESI-MS data suggested a molecular formula C₁₄H₁₂O₅ for the compound. Doublets of 1H each due to H-3 and H-4 at δ 6.20 and 7.99 together with two aromatic proton singlets, para to each other at δ 7.72 (H-5) and 6.67 (H-8) in the ¹H-NMR spectrum, indicated that the coumarin architecture remained unchanged during transformation. It also showed a small coupling constant (3.6 Hz) of two doublets at δ 4.74 and 3.63 due to H-4’ and H-3’ protons indicating their cis orientation. All spectroscopic data and specific rotation were in close agreement with those published for (−)-cis-decursidinol with 3’S, 4’(S) configuration. The compound was thus identified as (−)-cis-decursidinol (3).

Results and Discussion

Adopting the standard two stage procedure, decursin (1) was screened using ten fungal cultures. Of the six organisms which showed the ability to transform I, S. chrysospermum (ATCC 13378) was selected for scale up experiments anticipating better yields. The metabolites obtained were decursinol (2) and decursidinol (3).

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chrysospermem and several other fungal strains transform decursin (1) into (+)-decursinol (2) and (−)-cis-decursidinol (3). Decursinol and few other coumarins including decursin (1) are constituents of A. gigas. Compounds 1 and 2 together with decursin angelate show important biological activities. Trans-Decursidinol is also a natural product isolated from the roots of Peucedanum decursivum. Quantities of decursin and trans-decursidinol required to investigate the biochemical and pharmacological effects, including the pain relief applications are obtained by organic synthesis. However, except as reaction products there are no reports on the isolation and bioactivity of cis-decursidinol.

Since there are no reports on mammalian metabolites of decursin, the data on the microbial transformed products, (+)-decursinol (2) and (−)-cis-decursidinol (3) may be used for further pharmacological evaluation of decursin. They may also be used as analytical standards for detection in biological fluids.

The formation of more polar, phase I hydrolyzed (2) and oxidized (3) products may be viewed as an attempt to reduce the biological half-life of 1 to prevent its accumulation in the body.

Experimental

General Experimental Procedures IR spectra were measured in CHCl₃, on an ATI Mattson Genesis series FTIR spectrophotometer. UV spectra were run on a Hewlett Packard 8452A diode array spectrometer. Specific rotations were measured with a Jasco DIP-370 digital polarimeter. 1H- and 13C-NMR spectra were obtained on a Varian Unity Inova 600 spectrometer unless otherwise stated. HR-ESI-MS data were acquired using a Bruker GioApex 3.0.

Substrate Decursin (1) was isolated as a white gummy solid from the MeOH extract of the roots of A. gigas. Its authenticity was confirmed by physical NMR data.

Organisms and Metabolism Initial screening of decursin (1) was carried out with ten culture samples from the microbial collection of The National Center for Natural Products Research of The University of Mississippi. A two-stage screening procedure was followed using 25 ml medium α in 125 ml Erlenmeyer flasks. Compound 1 was added in dimethylformamide (0.5 mg/ml) to 24 h old stage II cultures and incubated for 14 d on a rotary shaker (New Brunswick Model G10-21) at 100 rpm. Precoated Si gel 60 F₂₅₄ TLC plates (E. Merck) with p-anisaldehyde as the spray reagent were used to monitor the reaction. Preparative scale fermentations were carried out in five 2 l flasks, each containing 100 mg of substrate in 500 ml medium α. Ethanol was used to extract the combined culture filtrates. Metabolites were isolated by column chromatography over silica gel. Culture and substrate controls were run along with the above experiments.

Microbial Transformation of Decursin (1) by S. chrysospermem

Ethanol extract of the combined culture filtrates was column chromatographed over silica gel (Si gel 230—400 mesh: E. Merck, 30 g, column diameter: 20 mm) with CHCl₃ gradually enriched with MeOH. Two compounds, 2 (15 mg) and 3 (10 mg) were isolated and identified by means of spectroscopic data.

Decursinol (2) was isolated as a white solid (15 mg, 3% yield). Rf 0.32 (hexane–EtOAc (3:2)); [α]D⁰ = +63.3° (c=0.11, MeOH). UV λₘₐₓ (MeOH) nm (log ε): 207 (4.71), 220 (4.30), 330 (4.39); IR νₖₐₓ (CHCl₃) cm⁻¹: 3440, 2970, 2334, 1717, 1626, 1539, 1133, 1067, 821. HR-ESI-MS m/z: 247.1003 [M+H]+ (Calcd for C₁₄H₁₅O₅, 247.0971).

Decursidinol (3) was purified as a white solid (10 mg, 2% yield). Rf 0.12 (hexane–EtOAc (3:2)); [α]D⁰ = −42.5° (c=0.11, CHCl₃). UV λₘₐₓ (MeOH) nm (log ε): 206 (4.49), 223 (4.15), 326 (4.09); IR νₖₐₓ (CHCl₃) cm⁻¹: 3489, 2922, 2854, 1708, 1623, 1558, 1460, 1384, 1288, 1147, 1197, 826. HR-ESI-MS m/z: 263.0990 [M+H]+ (Calcd for C₁₄H₁₅O₅, 263.0920).

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References and Notes