Penicillium sclerotiorum Catalyzes the Conversion of Herbertenediol into Its Dimers: Mastigophorenes A and B

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Herbertenediol was subjected to biotransformation by *Penicillium sclerotiorum*. Spectral data analysis of the converted metabolites revealed that the neurotrophic active compounds, mastigophorenes A and B, dimeric to the substrate were formed.

Key words *Penicillium sclerotiorum*; biotransformation; mastigophorene; dimerization

Mastigophorenes A—D (**1**—**4**) are dimeric herbertene-type sesquiterpenoids found only in the liverwort *Mastigophora diclados* (BRID.) NEES. They have usually been isolated with their precursor herbertenediol (**5**).[1,2] Our recent investigation of a liverwort sample collected from Madagascar, however, did not show any trace of mastigophorenes A and B but revealed the presence of **3**—**5**.[3] The remarkable neurotrophic activity of mastigophorenes A, B, and D has attracted considerable attention from synthetic chemists.[2] The synthesis of these compounds was started by Bringmann and coworkers[4] who used biomimetic oxidative dimerization of the protected monomeric precursor and atropoenantioselective synthesis *via* lactone methodology. Fukuyama *et al.*[5] used the same method to obtain a large amount of sample for biological evaluation. Even though no experimental evidence has been reported, it was obvious that the biosynthesis of **1** and **2** was from the oxidative phenolic coupling of (−)-herbertenediol.[2,6] To obtain structurally interesting and biologically active compounds and to evaluate the mechanism of introducing an oxygen functional group at a nonactivated carbon atom in organic compounds, biotransformation of secondary metabolites is a topic of systematic investigations in our laboratory. Carbon–carbon bond formation from the biotransformation of organic compounds is not common. Moreover, biotransformation using microorganism could give information on the enzymes involved in sesquiterpene biosynthesis. The present communication deals with the bioconversion of herbertenediol (**5**) into **1** and **2** by *Penicillium sclerotiorum*.

Results and Discussion

The *Penicillium* genus is fungi commonly growing as green or blue mold on decaying food and is used in making cheese and as a source of penicillin. *P. sclerotiorum*, was used for the first time in our laboratory for the biotransformation of organic compounds. After being identified, the microorganism was statically cultivated at 30 °C for 2 d in 200 ml of a medium (Czapek) containing 1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water H_2O (pH 7). After full growth of the microorganism, herbertenediol (**5**, 121 mg) was added to the medium and then the microorganism was shake-cultivated for a further 19 d at 30 °C. After filtration, metabolites from liquid–liquid extraction using EtOAc were separated on silica gel column chromatography to yield a mixture of mastigophorenes A and B together with the substrate.[7]

Compounds **1**[8] and **2**[8] (3.3% yield) were isolated as a mixture (ratio 3 : 1). Positive EI-MS exhibited a molecular ion peak at *m*/*z* 466, corresponding to the molecular formula $C_{30}H_{42}O_4$ as identified by HR-EI-MS. Inspection of the NMR spectral data revealed that the mixture contained herbertene-type compounds. The ^{1}H-NMR spectrum displayed two sets of signals due to four quaternary methyl groups (δ 1.93, 1.47, 1.21, and 0.79 and δ 1.92, 1.45, 1.20, and 0.79), together with an aromatic proton at δ 6.85 (s), signals of two hydroxyl groups at δ 5.57 (s) and δ 4.72 (s), and a multiplet at δ 2.66. The ^{13}C-NMR data showed signals similar to those of herbertenediol in which the signal the aromatic methine at δ_C 113.4 was replaced with a quaternary aromatic carbon (δ_C 117.1) and almost every carbon signal split by 0.07 to 0.25 ppm (see Experimental). The above data coupled with the molecular formula indicated that compounds **1** and **2** might be symmetrical dimeric compounds. One set of the ^{1}H- and ^{13}C-NMR signals were identical to those of mastigophorene **A**, whereas the other set was very similar to its **B** isomer. Both compounds were previously isolated from a sample of *Mastigophora diclados* collected from Borneo.[2] It is interesting to note that the ratio 3 : 1 of **1** and **2** after isolation was converted to 1 : 1 when the sample was kept at 0 °C for 3 d.

Liverworts are a rich source of dimeric compounds biosynthetically originating from their monomer by aryl–aryl bond formation.[6] These compounds are mastigophorenes (**1**—**4**), aquaticenol, and the antitumoral, antibacterial, and antimycotic active compounds, isoplagiochins.[6] Horseradish peroxidase is a well-known enzyme catalyzing oxidative coupling to synthesize dimeric compounds from the monomer.[9] The presence of the aryl–aryl bond formation-catalyzing enzyme in *P. sclerotiorum* could open up a new horizon in the biosynthesis of these metabolites as well as the synthesis of new and biologically active dimeric compounds. A similar enzyme could be present in the *M. diclados* sample from Borneo and this may be a difference between the sample

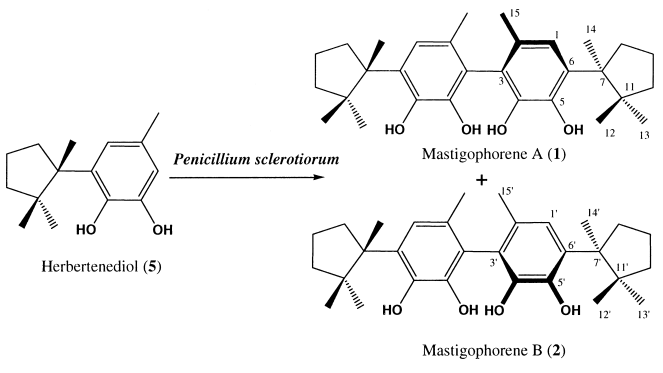

Fig. 1

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from Madagascar, since the former contains Mastigophorene A and B. Further investigations are needed.

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7) E-IMS *m*/*z*: 234 [M^+] (72%), 191 (5%), 178 (9%), 164 (56%), 151 (100%), and 137 (25%). ^{1}H-NMR ($CDCl_3$) δ: 0.76 (s, H-13), 1.18 (s, H-12), 1.41 (s, H-14), 2.22 (s, H-15), 2.60 (m, H-8a), 1.76 (m, H-8b), 1.67 (m, H-9ab and H-10ab), 6.68 (br s, H-1), 6.55 (br s, H-1), 5.35 (s, OH), 5.57 (s, OH). ^{13}C-NMR ($CDCl_3$): 20.2 (C-15), 21.1 (C-9), 22.7 (C-14), 25.4 (C-12), 26.8 (C-13), 39.2 (C-8), 40.9 (C-10), 44.8 (C-11), 51.0 (C-7), 113.4 (C-3), 121.9 (C-1), 128.3 (C-2), 133.4 (C-6), 140.8 (C-4), 143.3 (C-5).
8) EI-MS *m*/*z*: 466 [M^+] (100%), 423 (11%), 396 (11%), 384 (90%). HR-EI-MS 466.3086 ($C_{30}H_{42}O_4$) requires 466.3083. ^{1}H-NMR ($CDCl_3$), mastigophorene A (**1**) δ: 0.79 (s, H-13), 1.20 (s, H-12), 1.45 (s, H-14), 1.93 (s, H-15), 2.66 (m, H-8a), 1.75 (m, H-8b), 1.68 (m, H-9ab and H-10ab), 6.85 (s, H-1), 4.72 (s, OH), 5.57 (s, OH); ^{13}C-NMR ($CDCl_3$): 19.3 (C-15), 20.6 (C-9), 23.0 (C-14), 25.7 (C-12), 27.3 (C-13), 39.1 (C-8), 41.3 (C-10), 45.0 (C-11), 51.5 (C-7), 117.1 (C-3), 122.8 (C-1), 126.8 (C-2), 134.0 (C-6), 140.7 (C-4 and C-4′), 141.0 (C-5). Mastigophorene B (**2**) δ: 0.79 (s, H-13′), 1.21 (s, H-12′), 1.47 (s, H-14′), 1.93 (s, H-15′), 2.66 (m, H-8′a), 1.75 (m, H-8′b), 1.68 (m, H-9′ab and H-10′ab), 6.85 (s, H-1′), 4.72 (s, OH), 5.57 (s, OH); ^{13}C-NMR ($CDCl_3$): 19.4 (C-15′), 20.6 (C-9′), 22.7 (C-14′), 25.8 (C-12′), 27.4 (C-13′), 39.3 (C-8′), 41.4 (C-10′), 45.1 (C-11′), 51.5 (C-7′), 117.1 (C-3′), 122.9 (C-1′), 126.8 (C-2′), 134.1 (C-6′), 140.8 (C-4′), 141.0 (C-5′).
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