Trametes versatillis, a Fungus Highly Producing Eburicoic Acid

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Eburicoic acid was produced and isolated from the cultured mycelia of the fungus, *Trametes versatillis*. The weight rate of eburicoic acid in the dry weight of cultured mycelia of *Trametes versatillis* was about to 1%. *Trametes versatillis* can be expected as an important bioresource to supply eburicoic acid for its industrial exploitation.

Key words: *Trametes versatillis*, Eburicoic Acid, Cultured Mycelia

Introduction

Eburicoic acid is a triterpenoid acid naturally occurred and identified in 1950 (Gascoigen *et al.*, 1950). It is known as an important raw material of steroidal drugs. Recently, eburicoic acid and acetyl eburicoic acid have been revealed as potent apoptosis inducers after the cytotoxicity of these two compounds was evaluated on HL-60 human myeloid leukemia cells (Francisco *et al.*, 2004). Eburicoic acid occurs widely in the fruiting bodies of some mushrooms (Tai *et al.*, 1993; Shiono *et al.*, 2005; Sheth *et al.*, 1967), such as *Poria cocos*, *Leatiporus sulphureus* and *Fomes pinicola*. However, the weight content of eburicoic acid in such mushrooms is very low, usually below 0.1% in the dry weight. *Trametes versatillis* is a mushroom belonging to the family Polyporaceae, and up to date, there are no chemical reports on the fruiting bodies or cultured mycelia of this mushroom. We report herein on the fungus *Trametes versatillis*, which highly produces eburicoic acid by fermentation.

Results and Discussion

Eburicoic acid was isolated by column chromatography from the cultured mycelia of *Trametes versatillis*, and was identified by comparison with spectral data (including nuclear magnetic resonance spectroscopy and mass spectrometry) in the literature (Fried *et al.*, 1964). The eburicoic acid was up to 1% of the dry weight of the mycelia of *Trametes versatillis*. Eburicoic acid mainly existed in the cultured mycelia of *Trametes versatillis*, and it was also found a tiny trace of eburicoic acid in the fermentation broth.

Trametes versatillis is a favourable fungus, highly producing eburicoic acid, with several merits, that are producing more cultured mycelia, and short time for fermentation and sample preparation of culture media. Higher fungi in bioresource belong to the very productive biologically sources which produce a large and diverse variety of secondary metabolites. *Trametes versatillis* can be expected as such an important bioresource to supply eburicoic acid for its industrial exploitation.

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded on the Bruker DXR-500 instrument (Karlsruhe, Germany) at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR. Mass spectra were measured with a VG Autospec 3000 mass spectrometer (VG, England). Eburicoic acid was identified by comparison with spectral data in the literature.

Mushroom material

The fresh fruiting bodies of *Trametes versatillis* were collected at Ailao Mountain of Yunnan province, China, in October 2005. The botanical identification was made by Prof. Dr. Ji Dagan, Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (BJ-2005013) was deposited in the herbarium of the Kunming Institute of Botany.

Culture conditions for Trametes versatillis mycelia

After sterilizing the fruiting bodies of *Trametes versatillis*, a small piece of the stipe was inoculated onto a malt agar medium. The pure mycelia were subcultured for two weeks and grown in a 500 ml flask in 400 ml of medium containing 20 g dextrose, 150 g husked potato, 1.5 g $MgSO_4$, 3 g KH_2PO_4, 10 mg VB_1 in 1 l of distilled water.

Extraction procedure and separation of the EtOAc extract of the mycelia

After two weeks of culture (25 flasks), the mycelia were harvested with a nylon cloth and dried in an oven at 55 °C. The dry mycelia (96.5 g) of *Trametes versatillis* were comminuted and extracted with EtOAc (three times in a total of 1.5 l). The filtrates were combined and the organic solvent was removed under reduced pressure. The EtOAc extract (10.2 g) was subjected to chromatography over silica gel and then eluted with $CHCl_3$ to give fraction A and to $CHCl_3$/MeOH (98:2) to give fraction B. Fraction B (1.8 g) was subjected to further chromatography and eluted with petroleum ether/acetone (75:25) to yield eburicoic acid (960 mg).

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