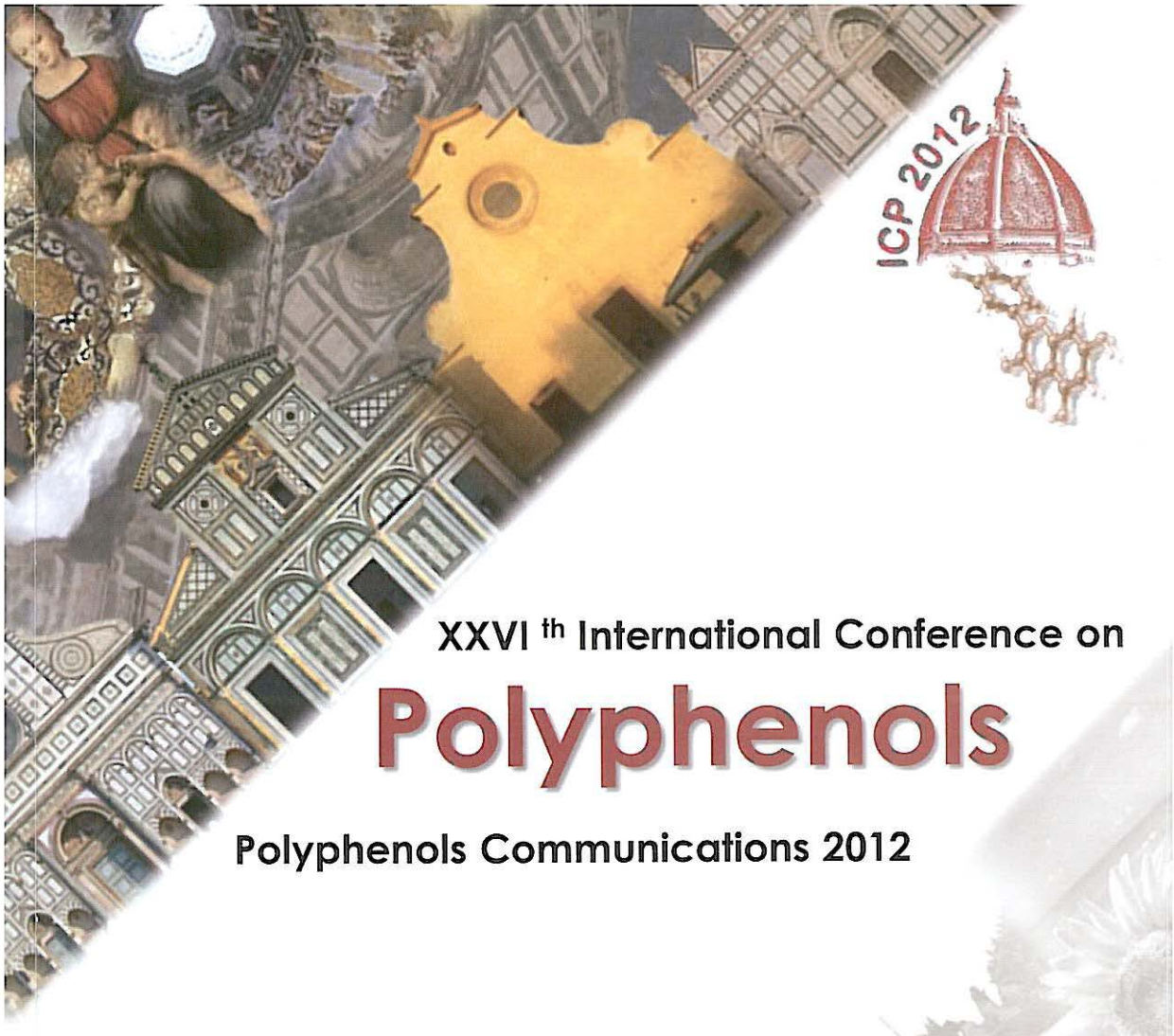

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Phenolic acids in the wild mushroom *Ganoderma lucidum* and synthesis of some possible metabolites

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Abstract. *Ganoderma lucidum* is one of the most extensively studied mushrooms due to its medicinal properties. *p*-Hydroxybenzoic acid was the most abundant phenolic acid found in this mushroom, after HPLC-DAD-MS analysis. In the present work we describe the synthesis of glucuronide and methyl derivatives of *p*-hydroxybenzoic acid, which are two of the main circulating metabolites found in humans. Their biological activity is going to be evaluated and compared to the parent compound, in order to understand the *in vivo* mechanism of action of phenolic acids, considering their metabolism.

Introduction.

Dietary phenolic compounds are widely considered to contribute to health benefits in humans. However, little is known about their bioactive forms *in vivo* and the mechanisms by which they may contribute toward disease prevention. Moreover, many studies on the biological effects of phenolic compounds have ignored the question of their achievable concentrations in the circulation after ingestion as well as the possibility of conjugation and metabolism [1].

Ganoderma lucidum is one of the most extensively studied mushrooms due to its medicinal properties. The phenolic extract of its fruiting body was characterized by us using HPLC-DAD-MS, being *p*-hydroxibenzoic (0.58 mg/100 g dw) and *p*-coumaric (0.38 mg/100 g dw) acids the main phenolic acids found in the extract. A related compound, cinnamic acid (0.28 mg/100 g dw), was also detected [2].

The present work aims at contributing to the knowledge of the mechanisms involved in the health-promoting properties of phenolic compounds, namely phenolic acids usually present in mushrooms. With that goal, circulating metabolites found in humans as sulfate, methyl and glucuronide derivatives are being prepared by chemical synthesis. Herein, we describe the synthesis of glucuronide and methyl derivatives of *p*-hydroxybenzoic acid, the most abundant phenolic acid found in *Ganoderma lucidum*.

Materials and Methods.

Glucuronidation of p-hydroxybenzoic acid

p-Hydroxybenzoic acid (0.050 g), acetobromo-α-D-glucuronic acid methyl ester (250 mg) and potassium carbonate (90 mg) were dissolved in 10 mL of DMF under argon and the mixture was stirred for 6 h at room temperature. The reaction mixture was diluted with 50 mL of EtOAc and then washed with water (7 × 10 mL). The organic layer was dried over $MgSO_4$ and the solvent was evaporated [3]. The product obtained was purified by a chromatographic column using a mixture of ether/petroleum ether (50:50, v/v) as eluent. The product was isolated as a white solid (30 mg, 10%), m.p.= 125.9-126.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.00 (s, 3H), 2.06 (s, 3H), 2.07 (s, 3H), 3.73 (s, 3H), 4.30 (d, *J* = 9.6 Hz, 1H), 5.29 (t, *J* = 9.2 Hz, 1H), 5.34 (dd, *J* = 9.2 and 7.6 Hz, 1H), 5.42 (t, *J* = 9.2 Hz, 1H), 5.94 (d, *J* = 7.6 Hz, 1H), 6.80 (d, *J* = 8.8 Hz, 2H), 7.81 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR: 20.47 (OAc), 20.53(OAc), 20.58 (OAc), 53.19 (OMe), 69.11 (CH), 69.94 (CH), 71.58 (CH), 72.77 (CH), 91.63 (CH), 115.41 (2xCH), 119.95 (C), 132.58 (CH), 161.28 (C), 163.99 (C=O), 167.53 (C=O), 169.45 (C=O), 169.56 (C=O), 169.91 (C=O). HRMS (ESI-TOF) calcd. for $C_{20}H_{22}O_{12}$ (M^+ + Na) 477.1004, found 477.0995.

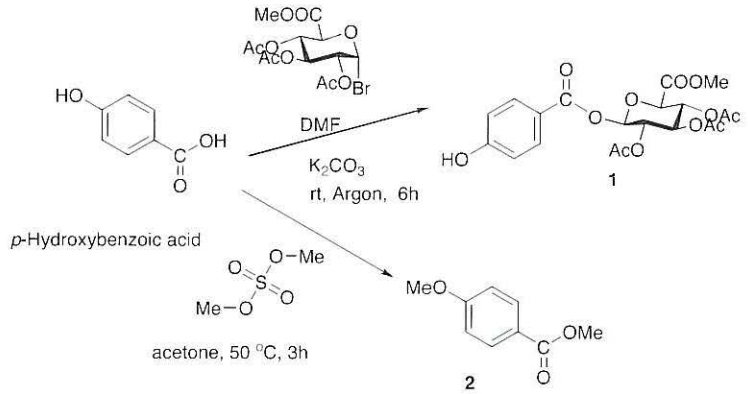
Methylation of p-hydroxybenzoic acid

p-Hydroxybenzoic acid (0.050g) , dimethyl sulphate (0.017 ml) and potassium carbonate (0.050 g) in acetone (10 mL) were stirred at 45-50°C for 3 h. The solvent was evaporated, the residue was suspended in water (3 mL) and extracted with ethyl acetate (3 × 3 mL) [4]. The combined ethyl acetate extracts were dried to give the product after precipitation with ether/petroleum ether as a beige solid (20 mg, 33%), m.p.= 48.6-49.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.79 (s, 3H), 3.82 (s, 3H), 7.04 (d, *J* = 9.0 Hz, 2H), 7.9 (d, *J* = 9.0 Hz, 2H). ^{13}C NMR: 51.81 (OMe), 55.50 (OMe), 114.03 (2×CH), 121.82 (C), 131.22 (2xCH), 163.12 (C), 165.89 (C=O). HRMS (ESI-TOF) calcd. for $C_9H_{10}O_3$ (M^+) 166.0630, found 166.0628.

Results and discussion

The *p*-hydroxybenzoic acid was reacted with acetobromo-α-D-glucuronic acid methyl ester (2 equiv.) to give the corresponding glucuronide ester **1** (Scheme) only in 10% yield. The reaction conditions need to be optimized to increase the yield compound **1**, and after that a deprotection will be carried out to obtain the corresponding *O*-glucuronide, which will be submitted to biological studies.

The methylation of the *p*-hydroxybenzoic acid using dimethyl sulphate (0.5 equiv.) gave the corresponding *p*-methoxyphenylester derivative **2** in 33% yield. This possible metabolite will also be submitted to biological studies.



Scheme. Methylation and glucuronidation of *p*-hydroxybenzoic acid.

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