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Structural identification of methyl protodioscin metabolites in rats' urine and their antiproliferative activities against human tumor cell lines

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ABSTRACT

Methyl protodioscin (MPD), a furostanol saponin, is a preclinical drug shown potent antiproliferative activities against most cell lines from leukemia and solid tumors. The metabolites of MPD in rats' urine after single oral doses of 80 mg/kg were investigated in this research. Ten metabolites were isolated and purified by liquid–liquid extraction, open-column chromatography, medium-pressure liquid chromatography, and preparative high-performance liquid chromatography. The structural identification of the metabolites was carried out by high resolution mass spectra, NMR spectroscopic methods including ^{1}H NMR, ^{13}C NMR and 2D NMR, as well as chemical ways. The 10 metabolites were elucidated to be dioscin (M-1), pregna-5,16-dien-3β-ol-20-one-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (M-2), diosgenin (M-3), protobioside (M-4), methyl protobioside (M-5), 26-O-β-D-glucopyrannosyl(25R)-furan-5-ene-3β, 22α, 26-trihydroxy-3-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside(M-6),26-O-β-D-glucopyranosyl(25R)-furan-5-ene-3β,26-dihydroxy-22-methoxy-3-O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyraooside (M-7), prosapogenin A of dioscin (M-8), prosapogenin B of dioscin (M-9), and diosgenin-3-O-β-D-glucopyranoside (M-10), respectively. M-1 was the main urinary metabolite of MPD in rats. Some metabolites showed potent antiproliferative activities against HepG2, NCI-H460, MCF-7 and HeLa cell lines in vitro.

1. Introduction

Methyl protodioscin (MPD, 1) is a furostanol biglycoside with the chemical name of 3-O-[α-L-rhamnopyranosyl-(1 → 2)-{α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranosyl]-26-O-[β-D-glucopyranosyl]-22-methoxy-25(R)-furost-5-ene-3β, 26-diol (Fig. 1). In the continuing efforts to seek bioactive components from the traditional Chinese herbal medicine, several steroid

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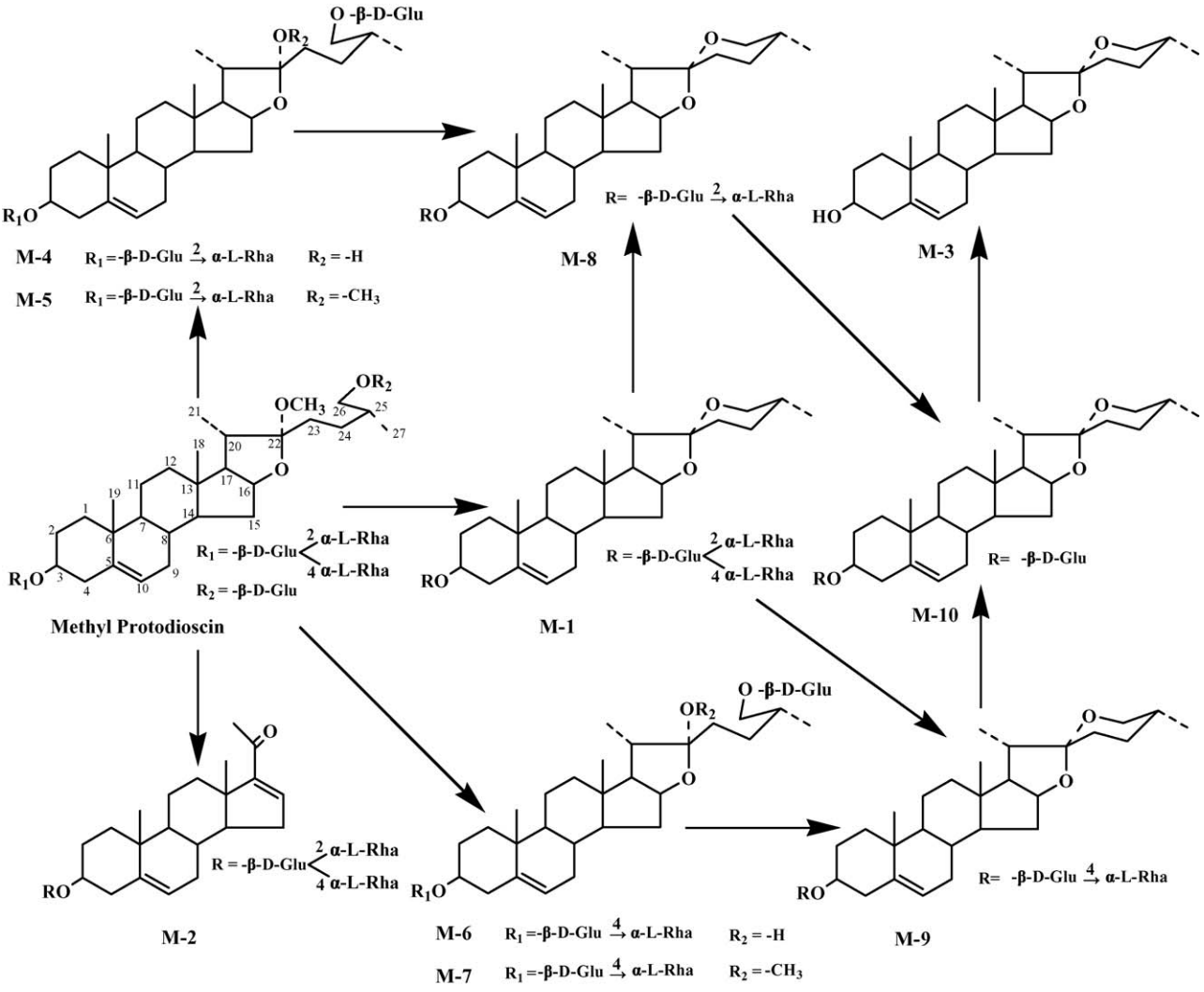

Fig. 1 – Proposed metabolic scheme of MPD in rats.

saponins with anticancer activities have been isolated from the rhizome of *Dioscorea collettii* var. *hypoglauca* (Dioscoreaceae), a traditional herbal remedy for the treatment of many carcinomas for centuries [1–3]. MPD showed strong activities against most cell lines from leukemia and solid tumors in the National Cancer Institute's (NCI) human cancer panel [4]. Except that widely existing in *Dioscorea* plants, MPD was also isolated from the rhizomes of *Smilax* [5,6], *Costus* species [7], etc. Methyl protodioscin has been synthesized for the first time from diosgenin through nine steps in one of our cooperative group [8], which guaranteed the material for the further research and applications in medicine. Biotransformation of MPD by *Cunninghamella elegans* and *Penicillium melinii* was investigated in the previous studies [9,10].

The preclinical investigations of MPD have been carrying out in our group, and the present work was attempted to get the detail information of its urinary metabolites in rats. Therefore, isolation, identification, and their antiproliferative activities were studied.

2. Materials and methods

2.1. Chemicals

MPD was isolated and purified from the fresh rhizome of *D. collettii* var. *hypoglauca* by Gexia Qu, Department of Natural Products Chemistry, Shenyang Pharmaceutical University, China. The isolation and purification procedures were briefly described below: 5.0 kg fresh rhizomes were cut into pieces and refluxed with 10 times volume 70% ethanol. The filtrate was evaporated under vacuum and removed all ethanol. The residue was then recovered with 2000 ml distilled water and subjected to D101 resin (Haiguang chemical factory, Tianjing, China) column (50 mm × 600 mm), and eluted with 3500 ml distilled water, 50% ethanol and 95% ethanol (v/v), respectively. The 95% ethanol elution (25.0 g) was further chromatographed by silica gel column (20 mm × 510 mm) and eluted with $CHCl_3$–MeOH–H_2O (80:20:1), and collected the fractions containing MPD according to their TLC results.