



BRIEF REPORT

5 α -Reductase Inhibitory Components as Antiandrogens From Herbal Medicine

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Abstract

We investigated medicinal plant sources with 5 α -reductase inhibitory activity. These compounds have been used in several remedies against androgen-dependent diseases including benign prostatic hyperplasia. The 50% ethanol extract of *Polygonum multiflorum* Thunb (Polygoni Multiflori Radix; Polygonaceae) showed potent 5 α -reductase inhibitory activity. The fraction responsible for this activity was purified, and the active constituent was isolated and identified as emodin, an anthraquinone compound. Although emodin showed considerably less potent inhibitory activity than riboflavin, the inhibitory activity of the compound was more potent than that of alizarin (1,2-dihydroxyanthraquinone), an anthraquinone-type positive control. Also, anthraquinone itself was substantially inactive against 5 α -reductase, indicating that the hydroxyl group on the structure of emodin is an important structural moiety for displaying inhibitory activity.

1. Introduction

Dihydrotestosterone, also known as 5 α -dihydrotestosterone acts as a more active androgen than testosterone in many tissues, including the prostate. Therefore, inhibitors of 5 α -reductase, which catalyzes the reductive conversion of testosterone to 5 α -dihydrotestosterone, may be useful in the selective treatment of androgen-dependent diseases, such as benign prostatic hyperplasia, male pattern baldness and acne [1]. Most developed 5 α -reductase inhibitors are steroidal compounds, which bind to steroid receptors, act as agonists or antagonists, and may produce various undesirable hormonal effects. Therefore, several 5 α -reductase inhibitory

active constituents from various plant sources have been isolated [2–11]. In this paper, we describe the inhibitory activity of the active component in a 50% ethanol extract of Polygoni Multiflori Radix (PMR; *Polygonum multiflorum* Thunb., Polygonaceae) on the activity of 5 α -reductase prepared from rat prostate.

2. Materials and Methods

2.1. General experimental procedures

Mass spectrometry spectra from the electron impact of 70eV were obtained with JMS AX505WA

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(JEOL Ltd., Tokyo, Japan) mass spectrometer. ^1H - and ^{13}C -NMR spectra at 400MHz and 100MHz were obtained on a JEOL JNM-AL400 spectrometer (JEOL Ltd.) with internal TMS as standard. Column chromatography was performed with silica gel (Merck & Co., Inc., Whitehouse Station, NJ, USA). TLC was performed on precoated Silica gel 60F₂₅₄ plates (Merck & Co., Inc.), and spots were visualized using UV light at 254nm. Riboflavin and alizarin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Anthraquinone was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Plant material

Plant samples of PMR were purchased from the Herbal Cosmeceutical Material Bank, Korea National Research Resource Center. Voucher specimens have been deposited at the herbarium of the Natural Products Chemistry Laboratory, Department of Herbal Pharmaceutical Engineering, College of Herbal Bio-industry, Daegu Haany University, Korea.

2.3. Preparation of extracts and isolation of active compound

The dried and chopped radix of PMR (500 g) was extracted with 50% ethanol (1.0L \times 3) at room temperature for 3 days. After filtration, the extract was evaporated under reduced pressure and lyophilized to give a 50% ethanol extract (135.5 g). The 50% ethanol extract was partitioned between ethyl acetate and water to give an ethyl acetate-soluble fraction (11.4 g) and an aqueous fraction, respectively. The ethyl acetate-soluble fraction from PMR was mixed with silica gel and then fractionated by silica gel column chromatography with *n*-hexane-ethyl acetate to give active Compound 1 (66.1 mg).

2.4. Enzymatic assay

Homogenate of the ventral prostate of male Sprague-Dawley rats was prepared and 5 α -reductase inhibition was measured using the methods previously reported [12].

3. Results

We investigated medicinal plant sources having 5 α -reductase inhibitory activity used in several remedies against androgen-dependent diseases, including benign prostatic hyperplasia. The 50% ethanol extract of PMR showed substantial 5 α -reductase inhibitory activity. A 500 $\mu\text{g}/\text{mL}$ solution of the dried 50% ethanol extract of PMR showed an 80.7% inhibition of the enzyme. The inhibitory activity of the 50% ethanol extract of this crude drug was superior to that of *Davallia mariesii* Moore (Davalliaceae) and *Panax ginseng* C.A. Meyer (Araliaceae), both of which have been used in several phytotherapeutic preparations in the treatment of androgen-dependent diseases. When the 50% ethanol extract was partitioned between ethyl acetate and water, the ethyl acetate-soluble portion exhibited inhibition of the enzyme. The portion was separated by repeated silica gel chromatography with the guidance of rat prostate 5 α -reductase inhibitory activity to give the active constituent, Compound 1, as an orange powder. Electron ionization mass spectrometry of the compound showed characteristic fragmentations at m/z 270 (M^+ , 100%), which can be assigned to peaks corresponding to an emodin moiety. Compound 1 was finally identified by inspection of ^1H - and ^{13}C -NMR spectra as emodin (Figure 1).

4. Discussion

In traditional Chinese medicine, PMR is prescribed to treat weak bones, premature graying of hair, hair loss, and also to tonify the kidneys, and balance a fragile yin. PMR is also described to have a laxative effect when taken internally. It has been shown to contain anthraquinones, such as emodin and stilbene glycosides, which are similar to resveratrol but have superior antioxidant activity [13]. However, the 5 α -reductase inhibitory effect of PMR is reported here for the first time. The isolated emodin was evaluated for inhibitory potency on rat prostate 5 α -reductase. Emodin inhibited the enzyme activity in a dose-dependent manner (Table 1). As shown in our data, although emodin showed considerably less potent inhibitory activity than riboflavin,

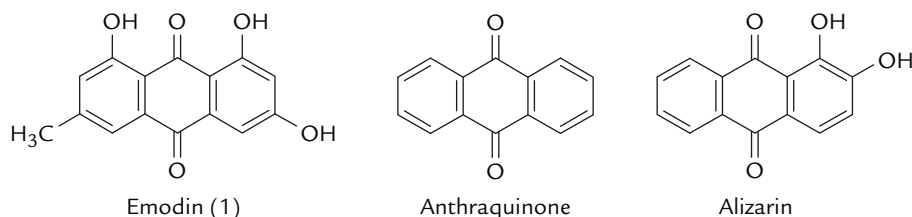


Figure 1 Chemical structures of emodin (1), from the radix of *Polygonum multiflorum*, anthraquinone and alizarin.

Table 1 IC₅₀ values of emodin (1) from the radix of *Polygonum multiflorum* and anthraquinone on rat prostate 5 α -reductase activity*

Compound	IC ₅₀ (μ M)
Emodin	40
Anthraquinone	> 1000
Alizarin	330
Riboflavin	1.6

*Value represents the mean of two experiments.

the inhibitory activity of the compound was more potent than that of alizarin, a naturally occurring 5 α -reductase inhibitor with an anthraquinone backbone [14]. Anthraquinone itself was substantially inactive against 5 α -reductase indicating that the anthraquinone part lacks inhibitory activity. This observation implies that the hydroxyl group in the structure of emodin is an important structural moiety for displaying inhibitory activity. Thus it may be necessary to examine the activity of a series of anthraquinone analogs to study the structure-activity relationship for enhancing inhibitory activity.

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