Comparative Anti-glycation and α-Glucosidase Inhibitory Studies of Metabolite, 9β,12β-Dihydroxy-9β,10α-pregna-4,6-diene-3,20-dione with Dydrogesterone

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Summary: Dydrogesterone (**1**) and its microbial hydroxylated metabolite 9β,12β-dihydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**2**) were screened for their anti-glycation and α-glucosidase inhibitory activities. Dydrogesterone (**1**) showed potent α-glucosidase inhibitory activity (IC_{50} = 95.6±1.3μM) when compared with 1-deoxynojirimycin used as standard (IC_{50} = 440.99±0.01 μM), while compound **2** was found to be weak inhibitor (IC_{50} = 824.3±6.1 μM). When evaluated for anti-glycation activities, both compounds **1** and **2** were inactive.

Key words: α-Glucosidase inhibition, dydrogesterone, 9β,12β-dihydroxy-9β,10α-pregna-4,6-diene-3,20-dione, anti-glycation

Introduction

Dydrogesterone (**1**) is similar to the progesterone. It is a synthetic hormone, which is widely used in the cure of menstrual disorders, threatened, endometriosis and habitual abortion. Dydrogesterone (**1**) is also used for postmenopausal hormone replacement therapy [1].

High concentration of glucose in "hyperglycemic condition" is mainly responsible of the glycation of different biomolecules (e.g. proteins, lipoproteins, and hormones), which cause diabetic complications [2, 3]. In glycation reaction, nitrogen bases at the proteins act as nucleophile, and attacks on the carbonyl center of the sugars without the catalytic assistance of any enzyme, resulting in the formation of Schiff base. These Schiff bases undergo rearrangement reaction and form Amadori product. In the last stage of glycation, Amadori products undergo sequence of dehydration, cyclization and condensation reactions and leads to the formation of advanced glycation endproducts (AGEs). Glycation of protein is associated with various pathological conditions particularly associated with diabetes and ageing etc. Therefore, there is need to explore new and safe anti-glycation, which can inhibit the formation of AGEs or make their formation a reversible reaction [4].

The small intestine possess a membrane bound enzyme known as α-glucosidase (EC 3.2.1.20), involved in the final stages of carbohydrate metabolism. α-Glucosidase catalyses the hydrolysis of disaccharides into monosaccharides. Inhibition of this enzyme can suppress the post prandial hyperglycemia, and therefore its inhibitors will be useful invention for the management of type II diabetes [5, 6].

In our previous study of microbial transformation of dydrogesteone (**1**) catalysed by *Rhizopus stolonifer*, which yielded 9β,12β-dihydroxy-9β,10α-pregna-4,6-diene-3,20-dione (**2**) (Fig. 1) [7]. The purpose of current paper is to describe comparative studies of anti-glycation and α-glucosidase inhibition of metabolite **2** with dydrogesterone (**1**).

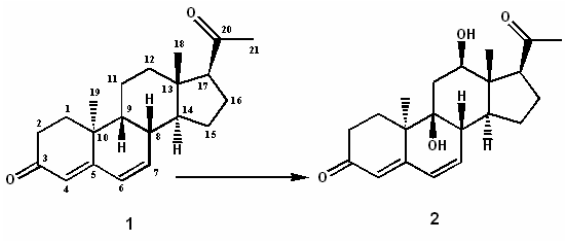

Fig. 1: Dydrogesterone (**1**) and its microbial hydroxylated metabolite **2**.

Results and Discussion

Dydrogesterone (**1**) and its metabolite **2** were subjected for their *in vitro* antiglycation activity. Both compounds **1** and **2** showed less than 50 % inhibition and were considered as inactive. Compound **2** (% inhibition = 23.35) showed higher antiglycation activity than substrate **1** (% inhibition = 15.66) as shown in Table-1.

Table-1: Antiglycation activity of compounds **1** and **2**.

S. Nos.	Compounds	Concentration (μM)	% Inhibition	IC_{50} (μM) ± S. E. M.
1	1	1000	15.66	NA
2	2	1000	23.35	NA
3	Rutin*	1000	86	294.5 ± 1.5

NA = Not active
S. E. M. = Standard error of the mean of three assays
* = Standard inhibitor for antiglycation bioassay

Dydrogesterone (**1**) and its metabolite **2** were also subjected for their α-glucosidase inhibitory activity. Compound **1** (i.e. substrate used for biotransformation) showed potent activity against α-glucosidase enzyme (IC_{50} = 95.6±1.3 μM) at 125 μM concentration when compared with 1-deoxynojirimycin used as standard (IC_{50} = 440.99±0.01 μM), while compound **2** (i.e. a biotransformed product) was found to be a weak inhibitor (IC_{50} = 824.3±6.1 μM) even at 1000 μM concentration as depicted in Table-2. Dose dependent graphs for both compounds **1** and **2** (% Inhibition versus concentration) are also shown in Fig. 2.

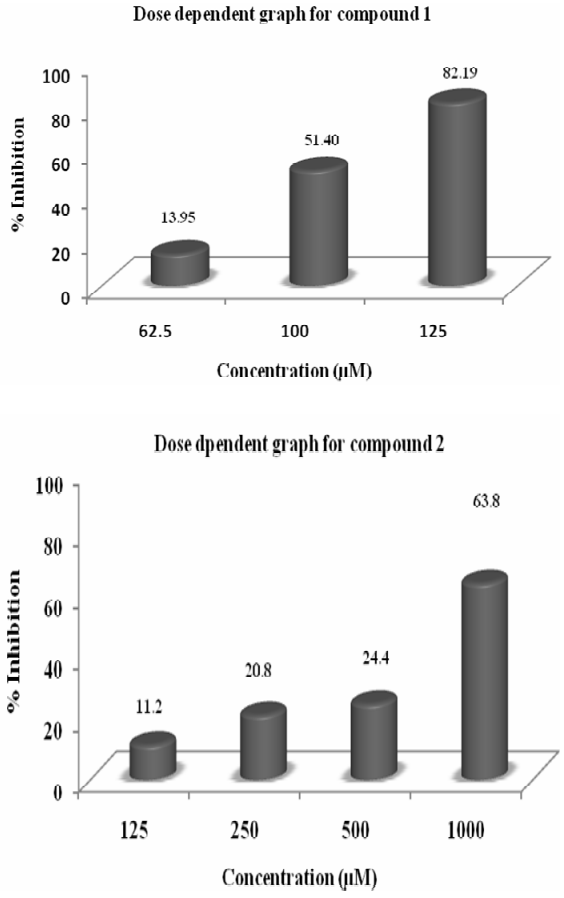

Fig. 2: Dose dependent graphs for α-glucosidase inhibitory activity of compounds **1** and **2**.

Table-2: α-Glucosidase inhibitory activity of compounds **1** and **2.**

S. No.	Compounds	Concentration (μM)	% Inhibition	IC_{50} (μM) ± S. E. M.
1	1	125	82.2	95.6±1.3
2	2	1000	63.8	824.3±6.1
3	1-Deoxynojirimycin*	1000	70.6	440.99±0.01

S. E. M. = Standard error of the mean of three assays
* = Standard inhibitor of the enzyme α-glucosidase

Materials and Methods

Rutin and methylglyoxal (MG) (40% aqueous solution) obtained from Sigma (Japan), BSA (Bovine Serum Albumin) was purchased from Merck Marker (Germany), sodium dihydrogen phosphate (NaH_2PO_4), sodium azide (NaN_3) and disodium hydrogen phosphate (Na_2HPO_4) were from Scharlau Chemie, S. A. (Spain), while dimethyl sulphoxide (DMSO) was from Fischer Scientific (UK).

In Vitro Antiglycation Assay

The total reaction volume of the assay was 200 μL. 10 mg/mL solution of BSA and 14 mM methylglyoxal was prepared in phosphate buffer (pH 7.4, 0.1 M) containing 30 mM NaN_3 as antimicrobial agent, while 1 mM solutions of test compounds were prepared in the DMSO. Reaction mixtures were comprised of 50 μL BSA, 50 μL methylglyoxal, pH 7.4 phosphate buffer (80 μL) and 20 μL test compound. The reaction mixture was incubated at 37 °C for nine days under aseptic conditions.

Each sample was examined after nine days of incubation for the specific fluorescence development (emission 420 nm; excitation 330 nm) on a microtitre plate reader against blank [8, 9]. Rutin was used as a positive control. The % inhibition of the AGE formation (for each compound) was calculated as the given formula:

% inhibition= (1- fluorescence of test sample/ fluorescence of the control group) × 100

In Vitro α-Glucosidase Inhibition Assay

In each experiment, 0.2 U/mL (concentration) α-glucosidase was used. The 20 μL enzyme along with 0.1 M phosphate buffer saline (100 μL, pH 6.8) was incubated with test compounds of various concentrations at 37 °C. The time of pre-incubation was specified at 15 min. After pre-incubation, the substrate (20 μL, 0.7 mM) was added to the mixture and the reaction was continued for 30 min at 37 °C. p-Nitrophenyl-α-D-glucopyranoside was used as substrate, while 1-deoxynojirimycin as positive control.

Enzymatic activity was quantified on a microtitre plate spectrophotometer at 400 nm by measuring the absorbance of *p*-nitrophenol [8]. The % inhibition of the formation of *p*-nitrophenol in the test sample *versus* control was calculated (for each compound) by using the given formula:

% Inhibition = 100 - (OD of test sample/OD of the control group) × 100

Statistical Analysis

All of the experiments were performed in 96-well plate and reactions were monitored in microtitre plate reader (SpectraMax M2, Molecular Devices, CA, USA). The results obtained from microtitre plate reader were analyzed by SoftMaxPro 4.8 and MS-Excel, and presented as means ± SEM from three experiments as indicated in the figure legend. EZ-FIT, Enzyme kinetics software (Perrella Scientific, Inc., USA) was used to determine IC_{50} values.

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