CHEMICAL STRUCTURE AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF TWO STEROID COMPOUNDS ISOLATED FROM THE STEM OF *MANGIFERA REBA*

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Title:
Cấu trúc và hoạt tính ức chế enzyme α-glucosidase của hai hợp chất steroid được phân lập từ thân cây quéo mangifera reba.

Từ khóa:
Mangifera reba, Anacardiaceae, ức chế enzyme α-glucosidase.

Keywords:
Mangifera reba, Anacardiaceae, α-glucosidase inhibitory activity.

TÓM TẮT: *Khảo sát thành phần hóa học cao ethyl acetate của thân cây Quéo (Mangifera reba) đã phân lập được 2 hợp chất steroid là β-daucosterol (1) và 6'-O-acetyl-β-daucosterol (2). Cấu trúc của các hợp chất đã được xác định dựa vào dữ liệu phổ cộng hưởng từ hạt nhân NMR và so sánh với các tài liệu tham khảo. Hai hợp chất trên đều thể hiện hoạt tính ức chế enzyme α-glucosidase với giá trị IC50 lần lượt là 207.3 và 83.5 μM so với chất đối chứng dương acarbose (IC50, 214.5 μM).*

ABSTRACT: *Study on the ethyl acetate extract from the stem of Mangifera reba have isolated β-daucosterol **(1)** and 6'-O-acetyl-β-daucosterol **(2)**. The chemical structures of these compounds were elucidated based on the NMR spectroscopic analysis and comparison with the literatures. These compounds showed more potent inhibitory activity, with IC_{50} values 207.3 and 83.5 μM respectively, than that of a positive control acarbose (IC_{50}, 214.5 μM)*

1. Introduction

According to the World Health Organization, diabetes is a chronic disease caused by a lack of insulin production by the pancreas or ineffective insulin leading to increased blood glucose. Diabetes can directly or indirectly cause disorders such as hyperglycemia, retinopathy, kidney failure, anemic heart disease, neuropathy, atherosclerosis, etc. Type 2 diabetes is an uncontrolled condition, multi cause metabolic disorder, causing dangerous complications. Controls type 2 diabetes by inhibiting α-glucosidase activity which can slow down the hydrolysis of carbohydrates and reduce blood sugar levels [1,2,3].

Currently, synthetic drugs such as acarbose, miglitol, voglibose, ... have the ability to inhibit carbohydrate hydrolytic enzymes such as α-amylase and α-glucosidase. However, these drugs are expensive, cause many side effects, and do not reduce diabetes complications[2]. Therefore, finding new drugs that are highly effective, have few side effects and are reasonably priced is an urgent issue.

The *Mangifera* genus is commonly grown in Vietnam with many different species. According to folk medicine, these plants have many uses such as laxative, diuretic, anti-inflammatory, analgesic, and anthelmintic.[4].

Many species in the *Mangifera* genus have had many publications on their chemical composition and biological activities such as antibacterial activity, anti-oxidation, and resistance to many cancer cell lines such as breast cancer on MCF-7, MDA-MB-435, MDA-N cell lines, kidney cancer on 786-0 cell line, colon cancer on SW-620 cell line, liver cancer on HepG2 cell line, hypoglycemia, ...[5,6,7,8].

Quéo or Xoài Quéo has the scientific name *Mangifera reba* P., belonging to the genus *Mangifera*, family Anacardiaceae, grows in tropical and sub-tropical regions of Asia. The trunk is large, 10-20 m high, young branches with edges. Leaves have oblong-lanceolate blades, 12-16 cm long, 3-5 cm wide, pointed tip, wide rounded base, lateral veins 18-22 pairs, thin, slightly prominent on upper surface. The stem is 0.1-0.25 cm long. The inflorescence at the top is 1.5 cm long, sessile, pyramidal, bristly, with upright branches bearing full flowers. Flowers are bisexual, sepals 5, 0.8 cm long, pointed triangular shape. The petals are longer than the sepals, curved, oblong, with 3 large glandular crests, up to half the length of the petals. The drupe is flat, 7-8 cm long, curved. The seed nucleus has many large veins[9,10].

As part of our continued study on the screening of medicinal plants for α-glucosidase inhibitory activity, we found that the CH_3OH-soluble extract of the stem of *Mangifera. reba* showed significant inhibitory activity with an IC_{50} value of 0.1 μg/mL, than a positive control acarbose[11].

Therefore, the study of extracting, isolating, determining the structure and testing the α-glucosidase inhibitory activity of substances that isolated from *Mangifera reba* to search for potential active ingredients in the treatment of diabetes has great scientific and application significance. Partial research results on the chemical composition and biological activities of the *Mangifera reba* have been published in two article[12,13].

2. Theoretical framework and Methods

2.1. General Procedures

The NMR spectra were taken on a Bruker Avance III 500 spectrometer (Bruker BioSpin AG) with tetramethylsilane (TMS) as internal standard. The UV-VIS absorbance was measured with a Shimadzu UV-1800 spectrophotometer (Shimadzu Pte., Ltd). Column chromatography was carried out using silica gel 60, 0.06–0.2 mm (Scharlau). Analytical and preparative TLC were carried out on precoated Kieselgel 60F254 (Merck KGaA). Other chemicals were of the highest grade available.

Nuclear magnetic resonance spectroscopy data were measured at the Central Analysis Laboratory, University of Natural Sciences - National University, City. Ho Chi Minh.

α-Glucosidase inhibitory activity was performed at the Pharmaceutical Chemistry Laboratory, Faculty of Chemistry, University

of Natural Sciences - National University, City. Ho Chi Minh.

2.2. Plant material

The stem of *Mangifera reba* was collected in the Ma Da Forest, Dong Nai province, Vietnam in March, 2014. The voucher samples have been deposited at Division of Medicinal Chemistry, Faculty of Chemistry, VNUHCM–University of Science. The plant was identified by Assoc. Prof. Tran Hop, Institute of Tropical Biology, Ho Chi Minh City.

2.3 Extraction and Isolation

The 6.0 kg dry stem powder of *Mangifera reba* was extracted respectively with n-hexane, ethyl acetate and methanol using shoxlet extraction method. Solvent was removed under reduced pressure to yield n-hexane fraction (51.5 g), ethyl acetate fraction (84 g) and methanol fraction (139.2 g).

The ethyl acetate fraction was subjected to a silica gel column, eluted with $CHCl_3$-CH_3OH (0→100 % EtOAc), to yield 10 fractions **QA** (0.9 g), **QB** (2.9 g), **QC** (0.9 g), **QD** (0.9 g), **QE** (1.7 g), **QF** (4.7 g), **QG (**30 g), **QH** (8.8 g), **QI** (11.9 g), **QJ** (7.7 g).

The fractions were subjected to column chromatography and thin layer chromatography to obtain pure compounds. From the **QF** fraction, compound **1** and compound **2** were isolated.

Compound **1** white powder, well-soluble in DMSO, ^{1}H (500 MHz, DMSO-d_6) NMR δ_H 0.64 (s), 0.82 (t, 6.9), 0.82 (d, 6.9), 0.89 (d, 6.9), 0.90 (d, 6.5), 0,94 (s), 3.01 (m), 2.89 (dt, 8.0, 4.5), 3.06 (m), 3.11 (td, 8.7, 4.8), 3.13 (dt, 8.0, 4.5), 3.40 (m), 3.60 (ddd, 12.0, 5.7, 2.0), 4.21 (d, 7.8), 5.31 (d, 2.3), signals for the presence of methylene groups and methine groups δ_H 1.2 - 2.5 ppm (Table 1). ^{13}C (125 MHz, DMSO-d_6) NMR spectrum showed 35 carbon signals. (Table 1).

Compound **2** white powder, well-soluble in DMSO, ^{1}H (500 MHz, DMSO) NMR δ_H 0.65 (s), 0.81 (d, 7.0), 0.84 (d, 7.0), 0.85 (t, 6.9), 0.91 (d, 6.5), 0.96 (s), 2.0 (s), 2.95 (t, 8.0), 3.08 (t, 8.0), 3.17 (dt, 8.0), 3.44 (m), 3.40 (m), 4.07 (dd, 12.0, 6.0), 4.22 (dd, 12.0, 2.0), 5.33 (d, 4.3), signals for the presence of methylenes and methines δ_H 1.2 - 2.5 ppm (Table 1). ^{13}C (125 MHz, DMSO-d_6) NMR spectrum showed 37 carbon signals. (Table 1).

2.4 α-Glucosidase inhibitory assay

3 mM *p*-nitrophenyl-α-D-glucopyranoside (25 μL) and 0.2 U/mL α-glucosidase (25 μL) in 0.01 M phosphate buffer (pH = 7.0) were added to the sample solution to start the reaction. Each reaction was carried out at 37 °C for 30 min and stopped by adding 0.1 M Na_2CO_3 (375 μL). Enzymatic activity was quantified by measuring absorbance at 401 nm.

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}}.100\%$$

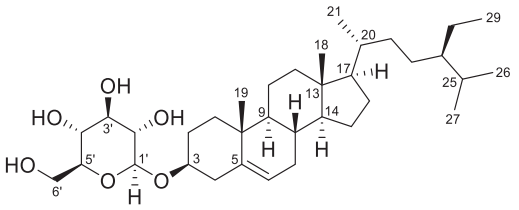
$A_{control}$: is the absorbance of control.

A_{sample}: is the absorbance in the presence of test substance.

One unit of α-glucosidase activity was defined as the amount of enzyme liberating *p*-nitrophenol (1.0 μM) per min.

The IC_{50} value was defined as the concentration of a α-glucosidase inhibitor that inhibited 50% of α-glucosidase activity.

Acarbose, a known α-glucosidase inhibitor, was used as a positive control.



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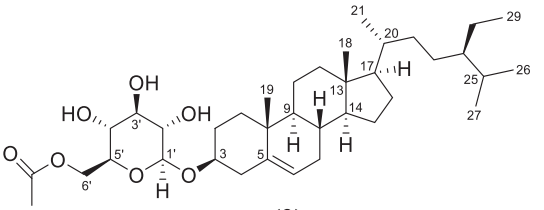

(2)

Figure 1. The chemical structures of compounds 1 and 2

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds 1 and 2

Position	Type C	1 (DMSO-d_6)		2 (DMSO-d_6)	
		δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	-CH_2-		36.8		36.8
2	-CH_2-		29.2		29.3
3	>CH(OR)	3.11 (td, 8.7, 4.8)	76.9	3.44 (m)	77.8
4	-CH_2-		38.2	2.37 (dd, 12.0, 3.4) 2.15 (t, 12.0)	38.5
5	>C=		140.4		140.5
6	=CH-	5.31 (t, 2.3)	121.2	5.33 (d, 4.3)	121.2
7	-CH_2-		31.4		31.9
8	>CH-		31.3		31.9
9	>CH-		49.6		49.6
10	>C<		36.2		36.2
11	-CH_2-		20.6		20.6
12	-CH_2-		38.3		38.5
13	>C<		41.8		41.8
14	>CH-		56.1		56.1
15	-CH_2-		23.8		23.8
16	-CH_2-		27.8		27.8
17	>CH-		55.4		55.4
18	-CH_3	0.64 (s)	11.6	0.65 (s)	11.7
19	-CH_3	0.94 (s)	19.1	0.96 (s)	19.1
20	>CH-		35.4		35.5
21	-CH_3	0.90 (d, 6.5)	18.9	0.91 (d, 7.0)	18.6
22	-CH_2-		33.3		33.3

23	-CH_2-		25.4		25.5
24	>CH-		45.1		45.1
25	>CH-		28.7		28.7
26	-CH_3	0.82 (d, 6.9)	19.8	0.84 (d, 7.0)	19.7
27	-CH_3	0.89 (d, 6.9)	19.0	0.81 (d, 7.0)	18.9
28	-CH_2-		22.6		22.6
29	-CH_3	0.82 (t, 6.9)	11.8	0.85 (t, 7.0)	11.8
1′	>CH(OR)	4.21 (d, 7.8)	100.8	4.27 (d, 8.0)	101.2
2′	>CH(OR)	2.89 (dt, 8.0, 4.5)	73.5	2.95 (t, 8.0)	73.4
3′	>CH(OR)	3.13 (dt, 8.0, 4.5)	75.9	3.17 (t, 8.0)	76.5
4′	>CH(OR)	3.01 (m)	70.1	3.08 (t, 8.0)	70.1
5′	>CH(OR)	3.06 (m)	76.7	3.40 (m)	73.4
6′	-CH_2(OR)	3.60 (dd, 11.6, 5.4) 3.40 (m)	61.1	4.22 (dd, 12.0, 2.0) 4.07 (dd, 12.0, 6.0)	63.8
6′-OAc		-	-	2.02 (s)	170.2 20.5

3. Results and discussion

Compound **1** white powder, well-soluble in DMSO. Thin layer chromatography with $CHCl_3$:MeOH (9:1) results in a circular spot that absorbs UV at 254 nm, visualizes with H_2SO_4 10% reagent, heats until the clearest color appears for a purple stain. The ^1H (500 MHz, DMSO-d_6) NMR spectrum showed signals of six methyl groups [δ_H 0.64 (s, H-18), 0.94 (s, H-19), 0.90 (d, 6.5, H-21), 0.82 (d, 6.9, H-26), 0.89 (d, 6.9, H-27), 0.82 (t, 6.9, (H-29)], one olefinic proton [δ_H 5.31 (d, 2.3, H-6)], one oxymethine proton [δ_H 3.11 (td, 8.7, 4.8, H-3)], signals for the presence of methylenes and methines δ_H 1.2 - 2.5 ppm.

Futhermore, ^1H NMR spectrum of compound **1** showed signals of a β-D-glucopyranose: one anomer proton [δ_H 4.21 (d, 7.8, H-1′)], three oxymethine protons, two oxymethylen protons [δ_H 2.89 (dt, 8.0, 4.5, H-2′), 3.13 (dt, 8.0, 4.5, H-3′), 3.01 (m, H-4′), 3.06 (m, H-5′), 3.60 (ddd, 12.0, 5.7, 2.0, H-6′a) and 3.40 (m (H-6′b)] (Table 1).

The ^{13}C (125 MHz, DMSO-d_6) NMR spectrum of compound **1** indicated the presence of 35 carbon, including seven methyl carbons [δ_C 11.6 (C-18), 19.1 (C-19), 18.9 (C-21), 19.8 (C-26), 19.0 (C-27)], two olefinic carbons [δ_C 140.4 (C-5), 121.2 (C-6)], one oxymethine carbon [δ_C 76.9 (C-3)], six carbons of a β-D-glucopyranose [δ_C 100.8 (C-1′), 73.5 (C-2′), 75.9 (C-3′), 70.1 (C-4′), 76.7 (C-5′), 61.1 (C-6′)] (Table 1).

The NMR data of compound **1** resembled those of ***β*-daucosterol**.[14]

Compound **2** white powder, well-soluble in DMSO. Thin layer chromatography with $CHCl_3$:MeOH (9:1) results in a circular spot that absorbs UV at 254 nm, visualizes with H_2SO_4 10% reagent, heats until the clearest color appears for a purple stain.

The ^1H NMR spectrum of **2** resembled that of **1**, including six methyl groups [δ_H 0.65 (s, H-18), 0.96 (s, H-19), 0.91 (d, 6.5, H-21), 0.84 (d, 7.0, H-26), 0.81 (d, 7.0, H-27), 0.85 (t, 6.9, H-29)], one olefinic proton [δ_H 5.33 (d, 4.3, H-6)], one oxymethine proton [δ_H 3.44 (m, H-3)], signals for the presence of methylenes and methines δ_H 1.2 - 2.5 ppm, signals of a *β*-D-glucopyranose: one anomer proton [δ_H 4.27 (d, 8.0, H-1′)], three oxymethine protons, two oxymethylen protons [δ_H 2.95 (t, 8.0, H-2′), 3.17 (dt, 8.0 H-3′), 3.08 (t, 8.0, H-4′), 3.40 (m, H-5′), 4.22 (dd, 12.0, 2.0, H-6′a) and 4.07 (dd, 12.0, 6.0, (H-6′b)], besides showed one acetoxy group δ_H 2.0 (s, $OCOCH_3$) (Table 1). The ^{13}C (125 MHz, DMSO-d_6) NMR spectrum of compound **2** indicated the presence of 37 carbons, including signals of *β*-sitosterol 3-*O*-*β*-D-glucopyranoside resembled that of **1,** besides showed signals of acetoxy group [δ_C 170.2, 20.5, $OCOCH_3$] (Table 1).

The NMR data of compound **2** resembled those of ***6′-O*-acetyl-*β*-daucosterol**.[15]

Compound **1** and compound **2** were tested for their *α*- glucosidase inhibitory activity. Acarbose, a known *α*-glucosidase inhibitor (IC_{50} = 214.51 μM), was used as the positive control in this study. These compounds showed potent activity with the IC_{50} values of of 207.3 and 83.5 μM, respectively

4. Conclusion

From the ethyl acetate extract of the stem of *Mangifera reba*, two steroid compounds were isolated, *β*-daucosterol **(1)** and 6′-*O*-acetyl-*β*-daucosterol **(2)**. The chemical structure of the compounds were determined by extensive NMR spectroscopy analysis and compared with data in the literature.

The results of testing the *α*-glucosidase inhibitory activity showed that both of these compounds showed stronger activity than the positive control acarbose.

The research results in this article and research results on the chemical composition and biological activity of the *Mangifera reba* that have been published previously[12,13] show that the chemical composition of *Mangifera reba* is very diverse, including lignan, neolignane, epoxylignan, diepoxy lignan, coumarin, chromone, megastigmane, steroids, simple phenolic compounds. Many isolated compounds showed stronger *α*-glucosidase inhibitory activity than the positive control.

The study has contributed to providing data on the chemical composition and biological activity of *Mangifera reba*.

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