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Isolation of Phenyl Propanoid Glycosides from *Allium tripedale* Trautv

Hamidreza Ghaffari[a], Masoud Sadeghi Dinani[b*]

[a] School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran
[b]Department of Pharmacognosy, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

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A B S T R A C T

Allium species are widely used plant species, which are consumed worldwide as raw vegetable, to make different dishes and as medicinal plants. As a member of this genus, *Allium tripedale* Trautv., locally called "Khargeriu, Anashq", is an important spicy edible *Allium* in "Zagros" region in the west and northwest of Iran which is used widely by local people as a spicy vegetable. Phytochemical investigation of underground part of the plant resulted to the isolation of two phenylpropanoid glycosides as the main phenolic constituents of chloroform-methanol extract of the bulbs. Chemical structure of afforded compounds were elucidated by comprehensive spectroscopic analyses including 1D and 2D NMR and MS as 3-(4-hydorxy phenyl)propyl β-D-Glucopyranoside (**1**) and coniferyl β-D-Glucopyranoside (coniferin) (**2**). Isolation and identification of phenylpropanoid derivatives from *A. tripedale* Trautv. is reported for the first time in this paper.

*Corresponding Author: Masoud Sadeghi Dinani, E-mail: m_sadeghi@pharm.mui.ac.ir

Introduction

Allium is the largest genus of the Amaryllidaceae family covering about 750 species including ornamental, edible and medicinal plants which are cultivated and collected all around the world with a more distribution in northern hemisphere [1-4].
Beside the use as common vegetables all over the world and also being an element of Mediterranean diet, *Allium* species are among medicinal plants which have been historically used for the treatment of many diseases specially cardiovascular diseases, hypercholesterolemia, obesity, diabetes, cataract, tumors and gastrointestinal tract complaints [2].
Phytochemically, *Allium* species are rich sources of important secondary metabolites involving steroidal saponin and sapogenins, flavonoids and organosulfur compounds [5,6] and numerous pharmacologically active compounds have been isolated from different *Allium species* in recent years. As a member of natural phenolic compounds, phenyl propanoid glycosides (PPGs) are valuable secondary metabolites widely distributed in different plant species including *Allium species* and have been demonstrated to possess different pharmacological activities specially in the treatment of hypertension, viral infections, fungal infections, tumors, cancer and immune system related disorders [7-11].
Honey garlic, *Allium tripedale* Trautv. is an spicy edible *Allium* of "Zagros" mountainous region in the west and northwest of Iran, wildly grows in this region and its strongly odorous leaves are used widely by local people as a raw vegetable and also to make different dishes [12]. The plant is locally called "Khargeriu" or "Anashq" and beside the culinary uses is also considered as a medicinal plant, traditionally has been used for the treatment of various infectious conditions [13]. As a part of our research program on different *Allium* species, phytochemical investigation of *A. tripedale* has been conducted and the current paper reports the isolation and identification of the main phenolic constituents have been isolated from the chloroform-methanolic extract of the plant.

Materials and Methods

General experimental procedures

Medium pressure liquid chromatography (MPLC) was performed by a Buchi Gradient System C-605 apparatus using glass columns of LiChroprep® RP-18 (25-40μm) and C-660 Buchi fraction collector. TLC performed on SiO2 plates with $BuOH:H_2O:CH_3COOH$ (60:25:15 v/v/v) (BAW) as a mobile phase and cerium sulfate in 2N H_2SO_4 and natural product (NP) as reagents for visualizing the spots.
HPLC was performed by Waters 515 apparatus equipped with a refractive index detector (Waters 2414) and UV detector (Waters 2487, λ : 254, 365 nm), using semipreparative C18 (Novapak®, 7.8 x 300 mm) and analytical C18 columns (Novapak®, 3.9 x 300 mm) in isocratic mode.
H and C NMR spectra recorded by Bruker 400MHz (H at 400 MHz and C at 100 MHz) spectrometer, using solvent signal for calibration (CD_3OD: δH=3.31, δC=49.0). Distortionless enhancement by polarization transfer (DEPT) experiments was used to determine the multiplicities of C NMR resonances.
2D heteronuclear multiple bond correlation (HMBC), optimized for $^{2-3}J_{CH}$ of 8 Hz, was used for determination of two and three bond heteronuclear $^{1}H-^{13}C$ connectivities, while 2D Heteronuclear Single-Quantum Coherence (HSQC), interpulse delay set for $^{1}J_{CH}$ of 130 Hz, was used for determination of one-bond heteronuclear $^{1}H-^{13}C$ connectivities. ESIMS spectra were prepared by Shimadzu LCMS 2010 EV, using methanol as the solvent.

Plant material

The whole plant of *A. tripedale* was collected from Khorram-Abad, Lorestan province on April 2013. The plant was identified by botanist and a voucher specimen (No. 3579) deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences, Iran.

Extraction and Isolation

Air-drided undergroud parts of *A. tripedale* were finely powdered by means of a mill and the powder (500 g) was extracted at room temperature in a four step extraction method with increasing solvent polarity using the solvents; hexane, chloroform, chloroform-methanol (9:1) and methanol. Extraction was done using maceration method, performing each step four times with 2 L of solvent under occasional stirring. The chloroform-methanol (9:1) extract was concentrated under vacuum, yielding a dried extract (12 g) which was then fractionated by MPLC on a RP-18 column (36*460 mm) using a linear gradient solvent system of H_2O to MeOH. Fractions were analyzed by TLC (SiO_2, BAW 60:15:25 v/v/v, reagents: cerium sulfate in 2N H_2SO_4 and natural product (NP)) and similar fractions were mixed together.

Based on TLC and preliminary HNMR analysis, the fraction 3 was considered to be rich in phenolic compounds which after concentration by rotary evaporator, subjected to HPLC using a semi preparative C18 column (Novapak®, 7.8x300 mm) and H_2O:CH_3OH (65:35) mobile phase in isocratic mode, resulted the pure compounds (**1**) (10 mg) and (**2**) (16 mg).

Results

Final purification of the main phenolic fraction resulted to the isolation of 2 pure phenyl propanoid glycosides. Structure elucidation of the isolated compounds were performed by comprehensive spectroscopic methods, resulted to the identification of coniferyl β-D-Glucopyranoside (Coniferin, Abietin) and 3-(4-hydroxyphenyl) propyl β–D-Glucopyranoside.

Characterization of compounds (1)

ESIMS spectra of compound (**1**) in the negative-ion mode showed a pseudomolecular ion peak at m/z 313 [M-H], while the HNMR spectrum exhibited 2 characteristic aromatic proton signals (δ_H 6.71 and δ_H 7.08), 2 sp^3 proton signals (δ_H 2.85 and δ_H 3.20) and an anomeric proton signal (δ_H 4.31) together with overlapped sp^3 proton signals (δ_H 3.22-3.81), suggesting the glycosilated aromatic nature for compound (**1**).

CNMR spectral analysis of (**1**) showed 13 carbon signals including 2 double height sp2 aromatic methine carbons (δ_C 116.13 and 130.91), 2 quaternary sp2 carbon signals (δ_C 130.76, 156.82), an anomeric carbon signal (δ_C 104.60) and 8 sp3 carbon signals (δ_C 19.80-78.25) which in agreement with MS and HNMR spectral data confirmed the glycosilated aromatic nature of compound (**1**) and suggested the molecular formula as $C_{15}H_{22}O_7$ (Table 2).

Finally by determination of low and high range ^1H-^{13}C connectivities through HSQC and HMBC experiments, and comparing the spectral data with those reported for similar compounds in the literature [14-16], the assignments including the attachment of glucose residue to C1' were confirmed and the chemical structure of (**1**) was defined as 3-(4-hydorxy phenyl)propyl β-D-Glucopyranoside (Fig. 1).

Characterization of compounds (2)

ESIMS spectra of compound (**2**) in the negative-ion mode showed a pseudomolecular ion peak at m/z 341 [M-H], while the HNMR spectrum exhibited the characteristic signals of aromatic protons (δ_H 6.28 -7.14) as well as an isolated doublet at δ_H 4.23 (2H, dd, *J*= 5.71, 1.26), an anomeric proton signal at δ_H 4.91(1H, d, *J*= 7.37) and overlapped proton signals of sugar residue (δ_H 3.33-3.79), altogether suggested the chemical structure of compound (**2**) as a glycosilated phenolic compound.

CNMR spectrum of compound (**2**) showed 16 carbon signals included those related to OCH_3 carbon (δ_C 56.74), C2-C6 carbon signals of sugar residue (δ_C 62.52-78.23), sp2 carbon signals (δ_C 111.42-150.91) and anomeric carbon signal (δ_C 102.77), which in agreement with MS and HNMR spectral data confirmed the glycosilated phenolic nature of compound (**2**) and suggested the molecular formula as $C_{16}H_{22}O_8$ (Table 2).

Finally by determination of low and high range ^1H-^{13}C connectivities through HSQC and HMBC

experiments, and comparing the spectral data with those reported for similar compounds in the literature [14,17-19], the assignments specially the attachment of OCH_3 to C3 and glucose residue to C4 were confirmed and the chemical structure of (**1**) was defined as coniferyl β-D-Glucopyranoside (Coniferin, Abietin) (Fig. 2).

Table 1. ^{1}H and ^{13}CNMR data of compound (1) and (2) (400 MHz, 100 MHz, CD_3OD)

Position	Compound (1)			Compound (2)		
	δ_C (mult)	**δ_H (int ,mult, J)**	**HMBC (C)**	**δ_C (mult)**	**δ_H (int ,mult, J)**	**HMBC (C)**
1	130.76 (C)	-	-	133.70 (C)	-	-
2	130.91(CH)	7.08 (1H,d,8.4)	3',3,6	111.42 (CH)	7.09 (1H,d,1.9)	1',3,6
3	116.13(CH)	6.71 (1H,d,8.4)	1,2,5	150.91 (C)	-	-
4	156.82(C)	-	-	147.65 (C)	-	-
5	116.13(CH)	6.71 (1H,d,8.4)	3', 2,3,6	117.96 (CH)	7.14(1H,d,8.4)	1',2,3,4,6
6	130.91 (CH)	7.08 (1H,d,8.4)	3',2,5	120.76 (CH)	6.97 (1H,dd,8.4,1.9)	1',2,5
1'	36.38 (CH_2)	3.20 (2H,t,8)	2',3'	131.30 (CH)	6.56 (1H,d,15.8)	3',1,2,6
2'	19.80 (CH_2)	0.95 (2H,dd,6.7,3.6)	1',3'	128.92 (CH)	6.28 (1H,dd,15.8,5.7)	1',3'
3'	71.68 (CH_2)	2.85 (2H,t,8)	1',2,6	63.73 (CH_2)	4.23 (2H,dd,5.71, 1.26)	1',2'
1″	104.60 (CH)	4.31 (1H,d,7.6)	2'', 1'	102.77 (CH)	4.91 (1H,d,7.37)	4,2''
2″	75.14 (CH)	3.48 (1H)[a]	1'',3''	74.92 (CH)	3.48 (1H) [a]	1'',3''
3″	77.98 (CH)	3.62(1H) [a]	2'',4'',5''	77.86 (CH)	3.60(1H) [a]	2'',4'',5''
4″	72.10 (CH)	3.22(1H) [a]	3'',5'',6''	71.36 (CH)	3.20(1H) [a]	3'',5'',6''
5″	78.25 (CH)	3.35(1H) [a]	4'',6''	78.23 (CH)	3.33(1H) [a]	4'',6''
6″	62.80 (CH2)	3.81(2H) [a]	4'',5''	62.52 (CH_2)	3.79(2H) [a]	4'',5''
OCH_3	-	-	-	56.74 (CH_3)	3.90 (3H,s)	2,3,4

[a] **overlapped with other signals**

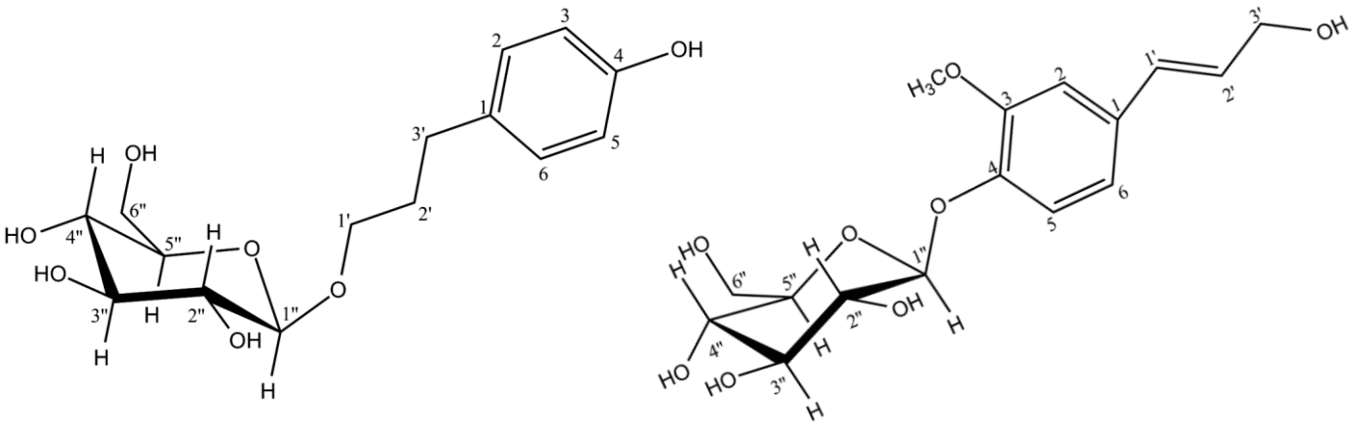

Fig. 1. Chemical structure of phenyl propanoid glycosides isolated from *A. tripedale.*

Discussion

Phytochemical investigation of the edible plant, *Allium tripedale*, resulted to the isolation of two phenyl propanoid glycosides from the underground parts of the plant, using the comprehensive spectroscopic methods, were identified as 3-(4-hydorxy phenyl)propyl β-D-Glucopyranoside (**1**) and coniferyl β-D-Glucopyranoside (coniferin) (**2**).

Phenyl propanoid glycosides are widely distributed phenolic compounds which have been isolated from numerous plant species including *Allium* species [7, 16-19] and have been shown to possess many pharmacological effects especially in the treatment of hypertension, viral and fungal infections, cancer and immune related disorders. They also have been demonstrated to bear significant antioxidative effects, mainly by inhibition of low-density lipoproteins oxidation through free radical scavenging and ion chelating activity [7-11,20].

According to their antioxidant activity, PPGs have also been suggested to be valuable in the prevention and treatment of a variety of diseases like cardiovascular and neurodegenerative disorders, atherosclerosis and diabetes type 2 [16, 20].

As a famous PPGs, Coniferin (Abietin) is an astringent glycoside which has been firstly isolated from coniferous plant species, while it has been isolated later from a variety of plant species mainly some *citrus* and *asparagus* species, comfrey and sugar beet . Beside the biological rule as a lignin precursor in many plant species, coniferin has been reported to show different pharmacological effects including anti-cough and anti-asthmatic properties [21]. Although different phenylpropanoid derivatives have been isolated from a vast range of plant families including Amaryllidaceae [7,16-19], there is just a report about the isolation and identification of ferulic acid-4-O-β-D-glucopyranoside from the underground parts of *Allium hookeri* [17], and to the best of our knowledge isolation of 3-(4-hydorxy phenyl)propyl β-D-glucopyranoside (**1**) and coniferyl β-D-glucopyranoside (coniferin) (**2**) from *Allium* species is reported for the first time in this study. The results could be used for

explanation of medicinal activities attributed to *A. tripedale* or to candidate the phenolic reached extract of the plant for new pharmacological studies based on the biological and pharmacological effects observed from different PPGs.

Conclusion

Phenyl propanoid glycosides are important secondary metabolites which have been reported to possess different pharmacological activities. Isolation and identification of phenyl propanoid glycosides from the underground parts of *A. tripedale* is reported for the first time in this study and could be used both as a chemical basis for explanation of medicinal effects of the plant and conducting pharmacological studies based on the medicinal activities of Phenyl propanoid glycosides.

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Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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