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Artigo

Chenoalbuside: an antioxidant phenolic glycoside from the seeds of *Chenopodium album* L. (Chenopodiaceae)

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ABSTRACT: In addition to three known phytoecdysteroids, a new phenolic glycoside (named, chenoalbuside) was isolated from the methanol extract of the seeds of *Chenopodium album*. While the structures of all phytoecdysteroids were elucidated by direct comparison of their spectroscopic data with published data, the structure of chenoalbuside was determined unequivocally by a combination of UV, MS and 1D and 2D NMR spectroscopic analyses. The antioxidant potential of the new compound was assessed by the DPPH assay, and the RC_{50} value was found to be 1.4 x 10^{-4} mg/mL.

Keywords: *Chenopodium album*, Chenopodiaceae, phytoecdysteroids, phenolic glycoside, chenoalbuside, anti-oxidant, NMR.

INTRODUCTION

Chenopodium album L., commonly known as 'pigweed', 'fat hen' or 'lambs-quarters' belongs to the family Chenopodiaceae. It is a woody annual, widely distributed in Europe, North America and Asia (Bailey, 1977; GRIN Database, 2005). Previous phytochemical studies on this plant furnished the presence of aldehyde (Tahara et al., 1994; Maruta et al., 1995), alkaloids (Horio et al., 1993; Cutillo et al., 2004), apocarotenoids (DellaGreca et al., 2004), flavonoids (Gohar; Elmazar, 1997), phytoecdysteroids (Dinan, 1992, Dinan et al., 1998; DellaGreca et al., 2005a), and an unusual xyloside (DellaGreca et al., 2005b). Various bioactivities, including antifungal (Tahara et al., 1994; Maruta et al., 1995), antipruritic, antinociceptive (Dai et al., 2002) and hypotensive (Gohar; Elmazar, 1997) properties, of crude extracts or isolated compounds from this plant were reported. As part of our on-going search for plant-derived antioxidants (Delazar et al., 2005; Delazar et al., 2004; Nahar et al., 2005; Sarker et al., 2005a,b; Sarker et al., 2003; Uddin et al., 2004), we now report on the isolation, structure elucidation and antioxidant activity of a new phenolic glycoside, named chenoalbuside (**1**), from the methanol extract of the seeds of *C. album*.

MATERIAL AND METHODS

General

UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV-Vis spectrometer (Agilent, Waldbron, Germany). CIMS (Chemical Ionisation Mass Spectrometry) analyses were performed in EPSRC Central Mass Spectroscopy Facility in Swansea, UK, on a Micromass Quattro II triple quadrupole instrument (Waters, Manchester, UK) in chemical desorption mode using ammonia as CI gas; mass accuracy was within 0.4 Da; CI source temperature 170 °C and electron energy 59eV. NMR spectra were obtained in CD_3OD using a Varian Unity INOVA 400 MHz NMR spectrometer. HMBC (Heteronuclear Multiple Bond Coherence) spectra were optimised for a long-range J_{H-C} of 7Hz. Preparative reversed-phase HPLC was carried out in a Dionex 580 HPLC system coupled with a UVD340S photo-diode-array detector and Gina50 autosampler (Gynkotek). A Luna C_{18} preparative column (21.2 x 250 mm, 10 µm) from Phenomenex (UK) was used. Sep-Pak Vac (Waters, USA) 10 g cartridge was used for pre-HPLC fractionation of the MeOH extract. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Fluka (UK).

Plant material

The seeds of *Chenopodium album* L. were purchased from B and T World Seeds, Sarl, Paguignan, France. A voucher specimen (PH005001 SDS) has been deposited in the herbarium of Plant and Soil Science Department, University of Aberdeen, Scotland, UK.

Extraction and isolation

The ground seeds (50 g) of *C. album* were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane and methanol (1.1 L each). All three extracts were concentrated using a rotary evaporator at a temperature not exceeding 50 °C. From the preliminary thin layer chromatographic analysis, it was obvious that

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the *n*-hexane and DCM extracts contained predominantly long-chain alkanes, and fatty alcohols, acids and esters, and therefore were not subjected to further phytochemical purification. The MeOH extract was subjected to pre-HPLC fractionation on a Sep-Pak cartridge eluted with a step gradient using 30, 60, 80 and 100% MeOH in water (250 mL each). Preparative reversed-phase HPLC (mobile phase: 0-50 min, linear gradient from 30 to 100% MeOH in water; 20 mL/min, detection at 220 nm) of the SepPak fraction, which resulted from the elution with 60% MeOH in water, produced previously reported phytoecdysteroids, 20-hydroxyecdysone (37 mg), 20-hydroxy-24-methylen-ecdysone (2.3 mg) and 20, 26-dihydroxyecdysone (5.1 mg; 25*R*:25*S* = 4:1) (Lafont and Wilson, 1996), and the structures of these compounds were determined by spectroscopic means. A similar reversed-phase preparative HPLC purification (mobile phase: 0-40 min, linear gradient from 25 to 55% MeOH in water; 20 mL/min, detection at 220 nm) of another SepPak fraction (30% MeOH in water) yielded the novel compound, chenoalbuside (**1**, 6.1 mg). The structure of this compound was determined conclusively by a series of spectroscopic data analyses.

Chenoalbuside (**1**): Brown amorphous; UV λ_{max} (MeOH) nm: 234, 296; CIMS *m/z*: 514 $[M+NH_4]^+$; ^1H and ^{13}C NMR: Table 1.

DPPH assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was used in this assay to assess the free radical scavenging (antioxidant) property of **1** (Kumarasamy et al., 2002; Takao et al., 1994). Quercetin, a well-known natural antioxidant, was used as a positive control. DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 μg/mL.

Qualitative Assay: Test compound (**1**) was applied on a TLC plate and sprayed with DPPH solution using an atomiser. It was allowed to develop for 30 min. The white spots against a pink background indicated the antioxidant activity.

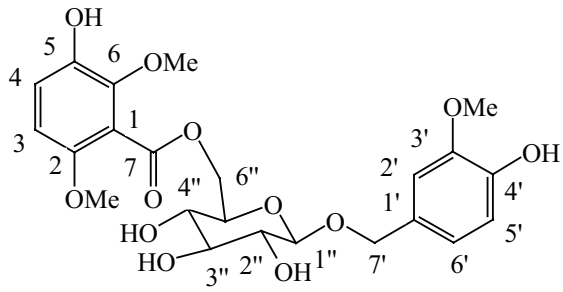
Quantitative Assay: For the quantitative assay, the stock solution of compound (**1**) was prepared in MeOH to achieve a concentration of 1 mg/mL. Dilutions

Table 1. ^1H NMR (400 MHz, coupling constant *J* in Hz in parentheses), ^{13}C NMR (100 MHz) and ^1H-^{13}C HMBC long-range correlations of chenoalbuside (**1**)

Carbons	Chemical shifts δ in ppm		^1H-^{13}C HMBC long-range correlations	
	^1H	^{13}C	^2J	^3J
1	-	116.9		
2	-	154.7		
3	6.87 d (8.5)	108.1	C-2, C-4	C-1, C-5
4	6.67 d (8.5)	111.0	C-3, C-5	C-2, C-6
5	-	145.6		
6	-	150.1		
7	-	164.8		
2-OMe	3.88 s	55.8		C-2
6-OMe	3.85 s	62.1		C-6
1’	-	129.0		
2’	7.18 d (1.8)	113.0	C-1’, C-3’	C-4’, C-6’, C-7’
3’	-	147.0		
4’	-	148.9		
5’	7.21 d (8.7)	115.6	C-4’, C-6’	C-1’, C-3’
6’	7.07 dd (8.7, 1.8)	121.9	C-1’, C-5’	C-2’, C-4’, C-7’
7’	6.05 d (10.0)	70.1	C-1’	C-1’’, C-2’, C-6’
	4.80 d (10.0)			
3’-OMe	3.89 s	56.2		C-3’
1’’	4.92 d (7.8)	101.9	C-2’’	C-7’, C-3’’, C-5’’
2’’	2.95 – 4.30*	73.1		
3’’	2.95 – 4.30*	77.0		
4’’	2.95 – 4.30*	71.0		
5’’	2.95 – 4.30*	75.7		
6’’	4.58 dd (12.1, 2.3)	64.9	C-5’’	C-7, C-4’’
	4.38 dd (12.1, 5.4)			

Spectra obtained in CD_3OD

*Overlapped peaks.



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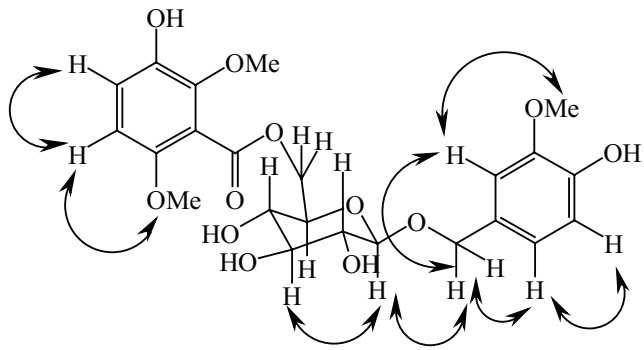

Figure 1. Key nOe interactions observed in the ^{1}H-^{1}H NOESY spectrum of **1**.

were made to obtain concentrations of $5x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$, $5x10^{-5}$, $5x10^{-6}$ $5x10^{-7}$, $5x10^{-8}$, $5x10^{-9}$, $5x10^{-10}$ mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance of these solutions was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control (quercetin).

RESULTS AND DISCUSSION

Reversed-phase preparative HPLC purification of the SepPak fractions obtained from the methanol extract of the seeds of *C. album* afforded three known phytoecdysteroids, 20-hydroxyecdysone, 20-hydroxy-24-methylen-ecdysone and 20, 26-dihydroxyecdysone (25*R*:25*S* = 4:1), and a novel phenolic glycoside, chenoalbuside (**1**). While the structures of the phytoecdysteroids were determined readily by direct comparison of their spectroscopic data with published data (Lafont; Wilson, 1996), the structure of **1** was elucidated by comprehensive spectroscopic data analyses, including UV, MS, 1D and 2D NMR.

The UV absorption maxima at 234 and 296 nm indicated the presence of a benzenoid structure with a conjugated carbonyl functionality. The CIMS analysis revealed the *pseudo*molecular ion, $[M+NH_4]^+$ at *m/z* 514, conducive to the molecular formula $C_{23}H_{28}O_{12}$. The ^{1}H and ^{13}C NMR spectra (Table 1) displayed signals, which could be attributed to a tetra- and a tri-substituted benzene rings, an ester carbonyl, an oxymethylene, three methoxyl groups, and a glucosyl unit. The ^{1}H and ^{13}C NMR data of **1** were similar to those for obtusaside, isolated from *Hypoxis obtusa* (Msonthi et al., 1990), with some striking differences in the signals associated with the tri-substituted benzene ring and the oxymethylene group. The ^{1}H-^{13}C HSQC experiment helped the identification of all carbons directly linked to the methane, methylene and methyl protons. The ^{1}H-^{13}C HMBC analysis (Table 1) provided information on the ^{2}J and ^{3}J ^{1}H-^{13}C long-range correlations. In this analysis, a ^{3}J correlation form H_2-6'' (δ_H 4.58 and 4.38) to the carbonyl (C-7, δ_C 164.8) of the 3-hydroxy-2,6-dimethoxybenzoate moiety. Similarly, a ^{3}J correlation from the oxymethyle H_2-7' (δ_H 6.05 and 4.80) of the 4-hydroxy-3-methoxy benzylalcohol moiety, to the glucose anomeric carbon (C-1'', δ_C 101.9), and a ^{3}J correlation from the glucose anomeric proton (δ_H 4.92) to the oxymethylene carbon (δ_C 70.1) confirmed the ether formation between C-1' and C-1''. While the position of all three methoxyl groups on the benzene rings were confirmed by the detailed ^{1}H-^{13}C HMBC analysis (Table 1), further confirmation on the placement of 2-OMe and 3'-OMe was obtained from the nOe interactions observed in the ^{1}H-^{1}H NOESY spectrum (Figure 1), respectively, between 2-OMe (δ_H 3.88) and H-3 (δ_H 6.87), and 3'-OMe (δ_H 3.89) and H-2 (δ_H 7.18). Thus the structure of this phenolic glycoside was determined conclusively as chenoalbuside (**1**). It is noteworthy that unlike obtusaside, where the oxygenations are at C-2' and C-5', the oxygenation on the benzyl alcohol moiety in **1** were at C-3' and C-4'. To our knowledge, chenoalbuside (**1**) is a novel natural product.

In the DPPH assay, chenoalbuside (**1**) showed

significant levels of free radical scavenging (antioxidant) activity compared to that of the positive control, quercetin. The RC_{50} values for **1** and quercetin were found to be 1.4 x 10^{-4} and 2.88 x 10^{-5} mg/mL, respectively. The antioxidant activity of **1,** like other natural phenolic antioxidants, *e.g.* flavonoids (Kumarasamy et al., 2004), is a consequence of the presence of the phenolic moieties in the structures. The antioxidant activity of phenolic natural products is predominantly owing to their redox properties, *i.e.* the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential (Kumarasamy et al., 2004).

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