Antibacterial Polyphenol from *Erodium glaucophyllum*

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Z. Naturforsch. **58 c**, 670–674 (2003); received November 25, 2002/February 28, 2003

Geraniin and gallic acid were isolated from the alcohol extract of the aerial parts of *Erodium glaucophyllum* (Geraniaceae). The identity of the compounds was verified through different physical and spectrometric methods. The antibacterial and antifungal activity of geraniin is presented.

Key words: Antibacterial Substances, Polyphenols, *Erodium glaucophyllum*

Introduction

The family Geraniaceae encompasses many promising plants from the medicinal point of view. Some of the family members are specified in the Chinese Pharmacopoeia and formulated in the Chinese herbal medicine such as lao-guan-cao formula. The drug consists of *Erodium stephanianum*, *Geranium nepalense* and *G. sibiricum* and is used to promote circulation in acute and chronic rheumatologic disorders and as detoxicant for enteritis and bacillary dysentery (Zhang *et al.*, 1995).

E. cicutarium was reported to exhibit antioxidative, antiviral, interferonogenic effects (Sroka *et al.*, 1995; Zielinska-Jenczylik *et al.*, 1988). Moreover, its extract exerts uterine haemostasis and stimulates the uterus to powerful contraction (Watt and Breyer-Brandwijk, 1962). From the chemical point of view, caffeine, tyramine, glutamic acid, choline, gallic acid, saponins, flavonoids and sugars were reported as common active constituents of *E. cicutarium* as well as of the family Geraniaceae (Hussein, 1985; Gibbs, 1974). However, the genera *Pelargonium, Geranium* and *Mansonia* were reported to contain tannins, the genus *Erodium*, was reported to be devoid of such constituents (Watt and Breyer-Brandwijk, 1962; Gibbs, 1974).

E. Glaucophyllum (L.) Aip. is a common herb in Nile valley, western Mediterranean coastal region and deserts (Tackholm, 1974), is known by Arabic names Kahkul, lesan Hamad, Kabshia, Ragma, Dahma, Murrar and Tamir and by English names Stork's bill, or Glaucus leaved stork's bill. It is used in folk medicine as oxytocic and astringent (Hussein 1985; Tackholm, 1974; Gibbs 1974). The absence of tannins in *Erodium* reported by Watt and Breyer-Brandwijk (1962) and Gibbs (1974) attracted the attention of the authors. So, the objective was to prove whether *Erodium*, as a genus belonging to family Geraniaceae, contains such constituent or not. Moreover, the use of some plants of the family for treatment of enteritis and bacillary dysentery (Zhang *et al.*, 1995) suggested the presence of antibacterial principles. In this paper the presence of tannins in *Erodium glaucophyllum* is demonstrated and proved to have antibacterial activity.

Results and Discussion

Compound **2** was obtained as a pale green crystalline material. Visualization of **2** on silica gel plates upon treatment with $FeCl_3$ (dark blue) suggested that it's a hydrolysable tannin. The compound exhibited strong absorption bands in the IR spectrum at 3407, 1725, 1611 cm^{-1} indicating the presence of -OH, CO and aromatic functions, respectively. Its UV spectra showed absorption maxima at 280 and 223 nm and exhibited a bathochromic shift (16 nm) on addition of $AlCl_3$ which was destroyed with HCl indicating the presence of vicinal hydroxyl groups (Latte and Kolodziej, 2000). Positive FAB-MS shows fragments having *m/z* 975 $(M+Na)^+$ and 991 $(M+K)^+$ calculated for molecular formula $C_{41}H_{28}O_{27}$ in addition to the mass fragment *m/z* 301 in the negative FAB spectrum, characteristic for hexahydroxydiphenic

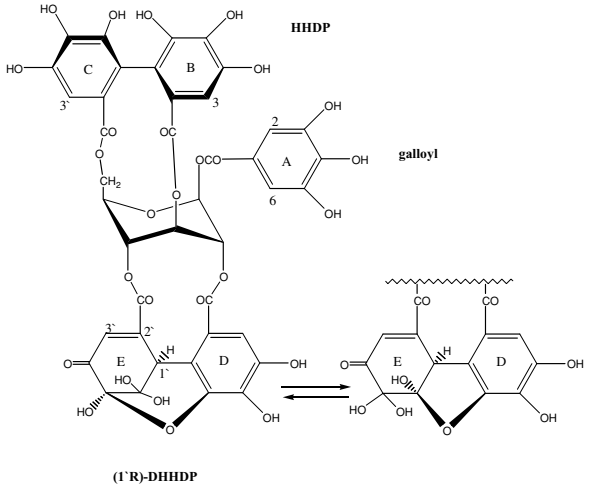

Fig. 1. Compound **2**: Geraniin.

acid (HHDP) (Latte and Kolodziej, 2000). ^{13}C NMR revealed the presence of 41 carbon signals (Table I), 5 of them were discriminated through DEPT experiments as aromatic C = O groups (δ 164–167), suggesting the presence of 5-galloyl residues. The downfield signal at δ 191.2 assigned to α–β unsaturated ketone and the upfield C-H at δ 45.0 suggested (1′*R*)-DHHDP. This conclusion was further confirmed by comparison of the chemical shifts of the other carbon atoms with reported data of related compounds (Yoshida *et al.*, 1994). ^{1}H NMR spectrum of **2** confirmed the previous conclusions. It displayed signals at δ 7.03 (2H, *s*), 6.45, 6.78 (each 1H, *s*), 7.05 (*s*), 4.88 (*s*) and 6.38 assigned for H-2, 6 gallic acid, H-3,3′ HHDP, H-3 dehydrohexahydroxydiphenic acid (DHHDP), H1′ ring-E and H-3′ ring-E of the DHHDP, res-

Table I: ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2**.

	Compound **2**		Compound **1**	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
Glucose				
1	6.35 (*d*, *J* = 3.0 Hz)	91.1		
2	5.33 (*d*, *J* = 3.6 Hz)	70.5		
3	5.39 (*br s*)	65.4		
4	5.20 (*br s*)	63.5		
5	4.70 (*t*)	72.5		
6	4.38 2H, (*m*)	63.0		
Galloyl				
1		118.5		121.9
2–6	7.03, 2H, (*s*)	109.3	7.05, 2H, (*s*)	110.3
3–5		145.6		146.4
4		143.9		139.6
7		165.2		170.4
HDDP				
1–1′		115.0, 116.2		
2–2′		122.5, 123.1		
3–3′	6.45, 6.78 (each 1H, *s*)	106.3, 107.6		
4–4′		144.7, 144.8		
5–5′		135.3, 136.2		
6–6′		145.6, 145.6		
7–7′		165.4, 167.4		
DHHDP				
Ring-D				
1		117.4		
2		114.8		
3	7.05 (*s*)	111.7		
4		142.9		
5		138.4		
6		144.2		
7		164.4		
Ring-E				
1′	4.88 (*s*)	45.0		
2′		152.3		
3′	6.38 (*s*)	128.0		
4′		191.3		
5′		90.9		
6′		95.7		
7′		164.1		

δ Value is expressed in ppm at 150 MHz in DMSO-d6.

pectively in addition to the aliphatic proton signals assignable to a fully acylated β-glucose in a $^1C_4$ conformation core (Amakura *et al.*, 1999) (Table I). This was confirmed by comparison of the different chemical shifts of the different protons with those of related compounds (Amakura *et al.*, 1999; Latte and Kolodziej, 2000; Jiang *et al.*, 2001). Accordingly, compound **2** was concluded to be geraniin. The compound was previously reported in *Geranii* herba (Kimura *et al.*, 1986), *Euphorbia humifusa* (Yoshida *et al.*, 1994), *Sapium sibeferum* (Cheng *et al.*, 1994), *Phyllanthus sellowianus* (Miguel *et al.*, 1996), *Acalypha hispida* (Amakura *et al.*, 1999), *E. pekinensis* (Hwang *et al.*, 2001).

Compound **1** ^{1}H NMR displayed a singlet at δ 7.02 assigned for two equivalent m-coupled aromatic protons. ^{13}C NMR of **1** displayed 5-signals in the aromatic region. Signal at δ 170.4 assigned for COOH group, two symmetric carbons at δ 110.3 and δ 146.4 for C-2, 6 and C-3, 5 respectively. The signal at δ 121.9 assigned to the carboxylated carbon atom and that at δ 139.6 for p-hydroxylated carbon. This pattern is fully comparable to that reported for gallic acid (Nawwar and Hussein, 1994). This was also confirmed by UV data, (Nawwar and Hussein, 1994) and by co-chromatography against a reference sample using chromatographic system A.

It's interesting to find tannins for the first time in the genus *Erodium*, family Geraniaceae in contrary to the previous report that the genus *Erodium* lacks such constituents (Watt and Breyer-Brandwijk, 1962; Gibbs, 1974).

Results of the antimicrobial activity of **2** against *Escherichia coli. Staphylococcus aureus* and *Candida albicans* (Table II) proved comparable activity in comparison to those of ampicillin (Epico, Egypt), gentamycin (Schering, USA) and mycostatin (Bristol-Mayer Squibb). The results of the MIC determination are shown in Table II. This confirmed the previous finding for the use of some plants of the family Geraniaceae for treatment of enteritis and bacillary dysentery (Zhang *et al.*, 1995).

From the biological point of view, geraniin was reported to exhibit inhibitory activity for chitin synthase II (Hwang *et al.*, 2001), inhibits the level of serum cholesterol, glutamate oxaloacetic acid transaminase, glutamate pyruvate transaminase and inhibits the formation of 5-lipoxygenase which should be a useful principle to find a natural drug for asthma and inflammation (Kumura *et al.*, 1986). Moreover, antihypertensive (Cheng *et al.*, 1994) and analgesic (Miguel *et al.*, 1996) properties were reported for geraniin.

Experimental

General

Mps uncorr., IR spectra in KBr discs, on a Buck Scientific INC. Infrared Spectrophotometer Model 500. UV in MeOH (Unicam SP 1800 ultraviolet spectrophotometer); NMR spectra were run at 600 MHz (^{1}H) and 150 MHz (^{13}C) in DMSO-d_6 using the solvent peak as internal standard (JEOL JNM A-600), the chemical shifts δ are expressed in ppm. Two-dimensional NMR experiments were preformed using standard programs. MS was obtained by positive and negative FAB^+ and FAB^- (JEOL JMS MS-700) Chromatographic systems: silica gel chromatoplate, EtOAc-MeOH-H_2O (10:1:0.5 v/v) solvent and vanillin/H_2SO_4 spray (system **A**), silica gel chromatoplate, EtOAc-MeOH-20% AcOH (10:1:0.5 v/v) solvent and NaOH 10% spray (system **B**), silica gel Rp-C18 plates, H_2O-MeOH (4:6 v/v) and NaOH 10% spray (system **C**).

Plant material

E. glaucophyllum was collected from King Saud University area, Riyadh, in the flowering stage, May 1996. Dr. Sultan UL-Abidin, taxonomist of

Table II: Minimum inhibitory concentration values for compound **2**.

Microorganism	MIC values			
	Compound **2** [mg/ml]	Ampicillin [mg/ml]	Gentamycin [mg/ml]	Mycostatin [I. U./ml]
Escherichia coli	2.5	–	0.125	–
Staphylococcus aureus	3.16	3.3	–	–
Candida albicans	1.99	–	–	12.5×10^3

MIC values determined by two-fold serial dilution assay. MIC values after a 48 h incubation at 37 °C.

the Faculty of Pharmacy KSU confirmed the identity of the plant. A voucher specimen has been deposited at the herbarium of the Pharmacognosy Department, KSU.

Extraction and fractionation: Powdered herb (450 g) was extracted with 2 liters EtOH 95% in a homogenizer for 30 min, filtered and the marc washed with 0.5 liter EtOH, vacuum filtered and the solvent was distilled off *in vacuo* to leave 28.5 g of a resinous brownish residue. The residue was dissolved in 100 ml water, partitioned with hexane, ethyl acetate and butanol (5 × 200 ml, each) to yield 5.3, 9.2, 7.8 mg, respectively. TLC screening of the extracts using chromatographic system **A** revealed a similar composition of the EtOAc and BuOH fractions. They were pooled, dissolved in EtOH and concentrated to syrupy consistency, then poured dropwise to 300 ml cooled ether with continuous stirring using a magnetic stirrer. The precipitated residue washed with ethyl ether, washings, and ether soluble fraction collected to afford 13 g after solvent evaporation. Preliminary antimicrobial screening of the hexane, ether-soluble, ether-insoluble and remaining aqueous mother solution fractions according to Mitscher *et al.* (1972) showed that the activity was concentrated in the ether-soluble fraction. Hence this fraction was subjected to bioautography (Hamburger and Cordell, 1987) on silica gel plates adopting system **A** for TLC [silica gel chromatoplate, EtOAc-MeOH-H_2O (10:1:0.5 v/v)] and using *B. subtilis* as test organism. Two inhibition zones were observed after 24 h of incubation at 37 °C.

Isolation of the active constituent

The ether soluble partitioned fraction (12 mg) was subjected to column chromatography (CC) over silica gel (70–230 mesh, 250 mg) and eluted with EtOAc- MeOH mixture of increased polarity; 250 ml fractions were collected. Fractions 1–6, eluted with EtOAc; 7–11 eluted with 5% mixture, 12–19 eluted with 10% mixture. Fractions 7–11 eluted with 5% mixture were found to contain a major single spot using system **B**. The compound was subjected to repeated CC, 100 g silica gel, isocratic elution with methylene chloride-acetone-methanol (70:25:5 v/v), 50 ml fractions were eluted, fractions 8–12 contained a single compound, R_f 0.86 (system **B**), as a creamy white powder from acetone, crystallized into needles from methanol, 25 mg, compound **1**.

Fractions 12–19 eluted with 10% mixture were similarly rechromatographed and from fractions 15–27 a single component; R_f 0.58 (system **B**) was detected. The residue after solvent evaporation was dissolved in acetone and precipitated with methylene chloride to yield 1 g of yellow powder. Repeated CC using Rp-C18 silica gel 75 g, and elution was done with water methanol mixtures (100 ml, each) of 10, 20, 30, 40, 50, 60, 70%. The eluate with 40% mixture afforded pale greenish crystals on concentration (650 mg, compound **2**), R_f 0.53 (system **C**).

Geraniin (**2**): Pale green needles, mp 238–240 °C, IR (KBr) ν cm^{-1}: 3407, 1725, 1611, 1515, 1445, 1336, 1195, 1086, 1054, 966, 910 and 875. UV λ_{max}: CH_3OH 280, 223; + CH_3ONa 330, 239; + NaOAc 281, 222; + NaOAc + H_3BO_3 303, 223; + $AlCl_3$ 296, 223; + $AlCl_3$ + HCl 279, 224. Positive FAB-MS 975 $(M+Na)^+$, 991 $(M+K)^+$, 797 (M-galloyl-2H), 783 (M-O-galloyl), 645 $(M\text{-}HHDP)^+$, 307 $(HHDP + 1)^+$, 154 $(galloyl + 1)^+$, negative FAB-MS 301 (HHDP). ^1H NMR and ^{13}C NMR data are listed in Table I.

Gallic acid (**1**): Needles from MeOH, mp. 251 °C (dec), UV λ_{max}: CH_3OH 218, 275 nm, IR (KBr) ν cm^{-1}: 3400, 1715, 1620, 1100, 737, ^1H NMR and ^{13}C NMR data are listed in Table I.

Acknowledgement

The pharmacist Shady K. George, M. Sc. Pharm., Microbiology Department, Faculty of Pharmacy, Mansoura University is highly acknowledged for running the antibacterial activity.

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