Pyrrolizidine alkaloids from Echium glomeratum (Boraginaceae)

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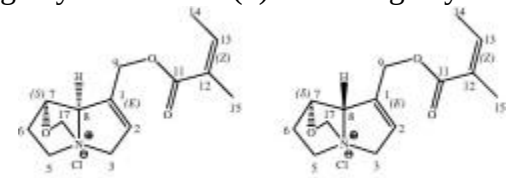
Abstract:
The methanolic extract of the whole plant of *Echium glomeratum* Poir. (Boraginaceae) has afforded five pyrrolizidine alkaloids, three that were (7*S*, 8*R*)-petranine (1), (7*S*, 8*S*)-petranine (2), and (7*R*, 8*R*)-petranine (3a) or (7*R*, 8S)-petranine (3b), comprising a tricyclic pyrrolizidine alkaloids subclass; and two that were known but to the species: 7-angeloylretronecine (4) and 9-angeloylretronecine (5). All compounds were tested against a human tumor panel for cytotoxicity; no activity was observed (EC_{50} values > 20 μg/ml).

Graphical abstract:
Five pyrrolizidine alkaloids were isolated from *Echium glomeratum* Poir. (Boraginaceae). Three of these were (7*S*, 8*R*)-petranine (1), (7*S*, 8*S*)-petranine (2), and (7*R*, 8*R*)-petranine (3a) or (7*R*, 8S)-petranine (3b), and two were known but to the species: 7-angeloylretronecine (4) and 9-angeloylretronecine (5).



(7*S*, 8*R*)-petranine (**1**) (7*S*, 8*S*)-petranine (**2**)

Keywords: *Echium glomeratum*; Boraginaceae; Pyrrolizidine alkaloids; Petranine; Angeloylretronecine

Article:

1. Introduction

Pyrrolizidine alkaloids are one of the more well-investigated classes of natural products. It has been estimated that the number of pyrrolizidine alkaloid-containing species is as high as 6000 or 3% of the world's flowering plants (Culvenor, 1980). Pyrrolizidine alkaloids are characteristic of the Asteraceae, Boraginaceae, Leguminaceae, and Orchidaceae families; 95% of species that contain pyrrolizidine alkaloids belong to one of these four families ([Bull et al., 1968], [Culvenor, 1980], [Hartmann and Witte, 1995], [Mattocks, 1986] and [Rizk, 1991]).

In general, pyrrolizidine alkaloids are derivatives of 1-methylpyrrolizidine, which consists of two fused five-membered rings with a bridgehead nitrogen. When hydroxylated, referred to as a necine, this structure can be either saturated or include a double bond between the 1 and 2 positions. Necine bases also may be *N*-methylated to form otonecine or oxygenated to produce *N*-oxides; the necine moiety can be esterified to necic acids, which vary significantly in structure ([Bull et al., 1968] and [Hartmann and Witte, 1995]). The polar nature of the *N*-oxide bond greatly enhances water solubility relative to the corresponding tertiary bases (Oberlies et al., 2004). Pyrrolizidine alkaloid *N*-oxides are somewhat unstable, readily converting into tertiary amines in the presence of weak reducing agents (Hartmann and Toppel, 1987). More than 300 pyrrolizidine alkaloid structures have been reported from the numerous pyrrolizidine alkaloid-containing plant species, wherein they accumulate typically as the *N*-oxides ([Bull et al., 1968], [Hartmann and Witte, 1995] and [Mattocks, 1986]).

As a chemical class, pyrrolizidine alkaloids are some of the leading plant-based toxins associated with harmful effects in both humans and animals. Their most prevalent toxicological features in vertebrates are hepatotoxicity, pneumotoxicity, mutagenicity, carcinogenicity and embryotoxicity and weak virustatic and antileukemic activities ([Bull et al., 1968], [Hartmann and Witte, 1995], [Mattocks, 1986], [Rizk, 1991], [Roeder, 1995], [Schmeller et al., 1997] and [Stegelmeier et al., 1999]). Interestingly, some semi-synthetic derivatives of pyrrolizidine alkaloids exhibit hypotensive, local anaesthetic, ganglionic and neuromuscular blocking, and antispasmodic activities (Atal, 1978).

As we have reported previously, the geopolitical borders of the Hashemite Kingdom of Jordan cover a wide range of elevation, from the lowest place on earth, 400 m below sea level at the shore of the Dead Sea, to plateaus of more than 1700 m above sea level near Jebel Rum (Alali et al., 2007). The varied topography and climate result in the junction of four biogeographical regions: the Mediterranean, the Irano-Turanean, the Saharo-Arabian, and the Tropical or Sudanian ([Al-Eisawi, 1998] and [Feinbrun-Dothan, 1986]), and these crossroads engender the country with diverse biodiversity that is largely unstudied ([Al-Eisawi, 1998] and [Feinbrun-Dothan, 1986]). In our ongoing research into under studied plants from throughout Jordan ([Alali et al., 2006a], [Alali et al., 2006b], [Alali et al., 2005] and [Alali et al., 2007]), *Echium glomeratum* Poir. was selected for phytochemical investigation.

Echium glomeratum Poir. is a member of the Boraginaceae (Al-Eisawi, 1998), a family of about 120 genera covering approximately 200 species (Watson and Dallwitz, 1992). Although several pyrrolizidine alkaloids have been reported from the genus *Echium*, they have not been reported previously from this species ([Betteridge et al., 2005], [Boppre et al., 2005], [El-Shazly et al., 1996a] and [El-Shazly et al., 1996b]). It is known as Tall Viper's-Bugloss, and locally as Sag Al-Hamam, and it is one of two species of *Echium* reported in Jordan, the other being *E. judaeum* Lacaita, which is known as Judean Viper's-Bugloss and to the local people as Lesan Al-Thoor (Al-Eisawi, 1998). Sag Al-Hamam was reported to be analgesic, diaphoretic, aphrodisiac, and used in snake bites (Karim and Quraan, 1986). *E. glomeratum* flourishes mostly in the northern and middle parts of Jordan, in the Mediterranean biogeographic region, where it can be found flowering between April and July. It is a perennial herb, with stiff bristly hairs and pink flowers arranged on recurved, dense spikes, along a tall stem-like inflorescence (Al-Eisawi, 1998). From the methanolic extract of the whole plant, five pyrrolizidine alkaloids were isolated and characterized. Three of these represent an interesting and new tricyclic pyrrolizidine alkaloids subclass, (7*S*, 8*R*)-petranine (**1**), (7*S*, 8*S*)-petranine (**2**), (7*R*, 8*R*)-petranine (**3a**) or (7*R*, 8S)-petranine (**3b**); the other two pyrrolizidine alkaloids were known but are new to the species: 7-angeloylretronecine (**4**) and 9-angeloylretronecine (**5**). The structures of all compounds were elucidated using a series of spectrometric and spectroscopic techniques. Moreover, all compounds were evaluated for cytotoxicity against a human cancer cell panel; no activity was observed (EC_{50} values > 20 μg/ml).

2. Results and discussion

As a result of the partitioning scheme, five fractions (A–E) were obtained. High concentrations of pyrrolizidine alkaloids were evident in the TLC of fraction B as detected by distinctive brown-orange spots after spraying with Dragendorff's reagent. Of the five pyrrolizidine alkaloids that were isolated from fraction B, compounds **1**–**3** showed a high degree of similarity in their ^{1}H, ^{13}C, DEPT-135, HSQC, HMBC, and mass spectral data, implying that these compounds may have an isomeric relationship.

Compound **1** (59.0 mg) was isolated as a white powder, m.p. 103–105 °C. The HRMALDITOFMS data (obsd *m/z* 286.11966 for $[M + H]^+$) revealed the molecular formula as $[C_{14}H_{20}ClNO_3 + H]^+$ (*m/z* 286.1204), which corresponded to 6° of unsaturation. The chemical shift values for the base ring protons were quite distinctive of a necine type pyrrolizidine ring (Logie et al., 1994). The complete ^{1}H, ^{13}C, DEPT-135, COSY, and HSQC data sets are shown in Table 1.

Table 1.

^{1}H-, ^{13}C-, DEPT-135, HMBC, and COSY NMR data (in $CDCl_3$) for compound **1**

Position	δ_C	DEPT	δ_H, multiplicity (J in Hz)	HMBC (H → C)	COSY (H → H)
1	134.4	C			
2	121.1	CH	5.82 bs	8	3a, 3b, 8, 9
3a	70.9	CH_2	4.55 d (15.5)		2, 3b, 8, 9
3b			4.77 d (15.5)		2, 3a, 9
5a	63.4	CH_2	4.09 m		5b, 6a
5b			4.35 m		5a, 6b
6a	34.7	CH_2	2.48 m		6b, 5a, 7
6b			2.70 m		6a, 5b, 7
7	70.2	CH	4.92 bs		6a, 6b, 8
8	87.7	CH	5.36 bs		2, 3a, 7, 9
9	59.8	CH_2	4.87 bs	1, 2, 11	2, 3a, 8
11	167.5	C			
12	126.9	C			
13	140.3	CH	6.18 qq (1.5, 7)		14, 15
14	16.1	CH_3	2.00 dq (1.3, 7.3)	12, 13	13
15	20.6	CH_3	1.90 d (1.5)	11, 12, 13	13
17a	68.8	CH_2	5.42 d (10)		17b
17b			5.75 d (10)		17a

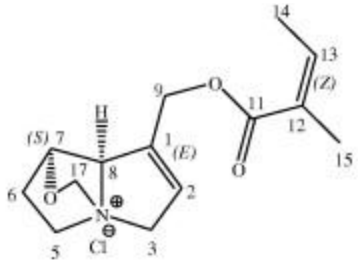
Characteristic signals for a 1,2-unsaturated pyrrolizidine alkaloid with a necic acid esterified at C-9 were evident. The ^{1}H NMR spectrum (Table 1) showed signals assignable to four methine protons at δ_H 5.82 (H-2), 4.92 (H-7), 5.36 (H-8), and 6.18 (H-13), four methylene protons at δ_H 4.55 (H-3a), 4.77 (H-3b), 4.09 (H-5a), 4.35 (H-5b), 2.48 (H-6a), 2.70 (H-6b), and 4.87 (H_2-9), and two methyl protons at δ_H 2.00 (H_3-14), and 1.90 (H_3-15). In the ^{13}C and DEPT-135 NMR spectra, signals were observed for: two quaternary carbons at δ_C 134.4 (C-1) and 126.9 (C-12); four tertiary carbons at 140.3 (C-13), 121.1 (C-2), 87.7 (C-8), and 70.2 (C-7); four secondary carbons at 70.9 (C-3), 63.4 (C-5), 59.8 (C-9), and 34.7 (C-6); two primary carbons at 20.6 (C-15) and 16.1 (C-14); and a carbonyl carbon at 167.5 (C-11). Examination of the COSY data revealed the following correlation system H-2/H_2-3/H-8/H-7/H_2-6/H_2-5, which in conjunction with other data for the olefinic methine group (δ_H/δ_C 5.82/121.1, H-2/C-2) and the olefinic quaternary carbon (δ_C 134.4, C-1), confirmed the presence of a necine base with a double bond between the 1 and 2 positions (Logie et al., 1994).

The substituent at C-9 was found to be an angeloyl moiety. The single proton at δ_H 6.18 ppm (H-13; quartet of quartet splitting pattern) displayed J values of 7 Hz and 1.5 Hz for coupling with the vicinal and allylic methyl groups at δ_H 2.00 ppm (H_3-14) and δ_H 1.90 ppm (H_3-15), respectively (Roeder et al., 1991). Moreover, the strong correlation observed in the COSY spectrum between H_3-14 and H-13 and the HMBC correlations between H_3-15 and C-11, C-12, and C-13 provide additional evidence for the presence of the double bond between the 12 and 13 positions; these data are in good agreement with a separate investigation of pyrrolizidine

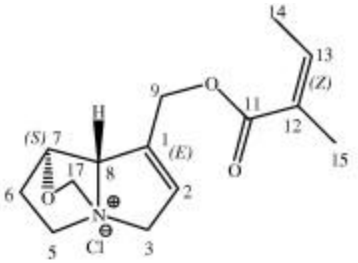
alkaloids that also contained an angeloyl moiety (Kim et al., 2001). Esterification at the C-9 position was confirmed by an HMBC correlation between H_2-9 and C-11.

The aforementioned assignment accounted for 5 of 6 the degrees of unsaturation. From the ^{1}H, ^{13}C and DEPT-135 NMR spectra, two coupled doublets, each corresponding to a single proton, and a methylene carbon with a somewhat unusual chemical shift value were observed, and, thus, a bicyclic bridge structure between the oxygen at C-7 and the nitrogen atom of the base ring via a methylene group was proposed. Examination of the COSY data revealed only geminal couplings for the H_2-17 protons. The proton and the carbon chemical shifts values of the methylene group H_2-17/C-17 ($\delta_{Ha}/\delta_{Hb}/\delta_C$ 5.42/5.75/68.8) supported its connectivity to an oxygen atom and to a quaternary carbon or heteroatom. Also, the chemical shift values of C-3, C-5, and C-8 (70.9, 63.4, and 87.7, respectively) were shifted downfield relative to most necine bases, and this indicated that the nitrogen was quaternary and positively charged. Presence of a chloride anion in the molecular formula of the compound was confirmed from the ESI-MS data, which revealed two molecular isotopic ion peaks, *m/z* 286 $[M + H]^+$ (100%) and *m/z* 288 $[M + H]^+$ (37%), where the later corresponded to the ^{37}Cl isotopic peak. Noteworthy, we cannot exclude the possibility that this chloride anion could be an artifact that may be coming from the extraction procedures or from the NMR $CDCl_3$ solvent.

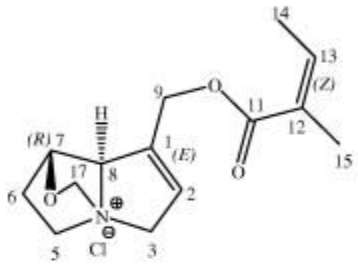
Despite considerable effort to form a crystal and due to the paucity of sample, it was not possible to use X-ray crystallographic analysis to determine the absolute configuration of the two asymmetric centers at positions 7 and 8. Hence, ROESY analysis was used to determine the relative configuration of these two chiral centers, which were proposed as 7*S* and 8*R*. This was supported by through space correlations between H_2-17 and H-3b, and between H_2-17 and H-8 (Fig. 1). The name (7*S*, 8*R*)-petranine was ascribed to this novel tricylclic pyrrolizidine alkaloid.



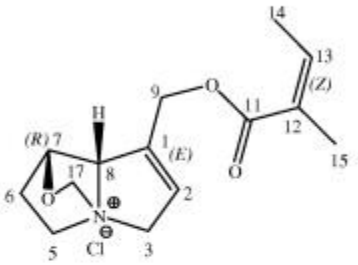
(7*S*, 8*R*)-Petranine (**1**)



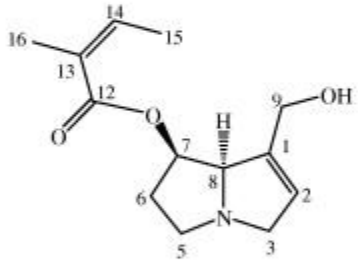
(7*S*, 8S)-Petranine (**2**)



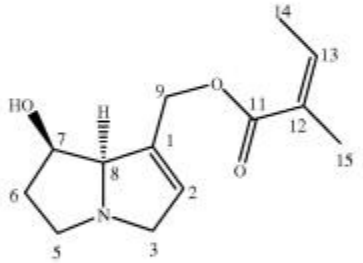
(7*R*, 8*R*)-Petranine (**3a**)



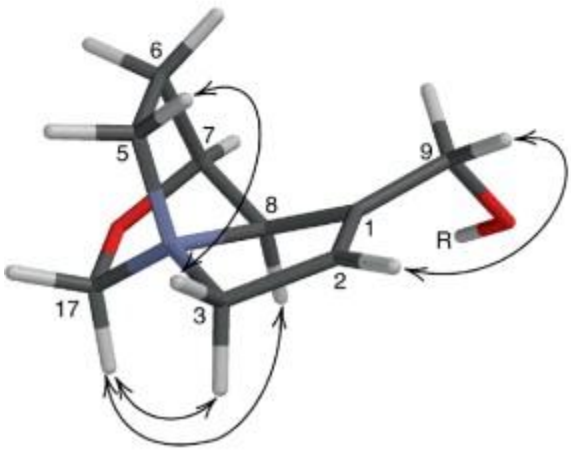
(7*R*, 8*S*)-Petranine (**3b**)



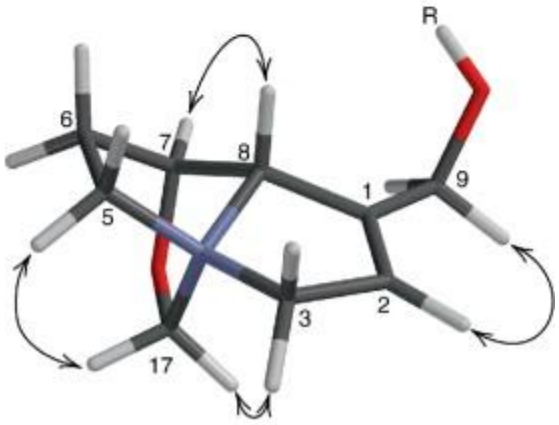
7-Angeloylretronecine (**4**)



9-Angeloylretronecine (**5**)



Compound **1**



Compound **2**

Fig. 1. Key ROESY correlations to distinguish between compounds **1** and **2**, where *R* represents the angeloyl side chain.

Compound (**2**) (35.0 mg) was obtained as white solid. The molecular formula was determined as $[C_{14}H_{20}ClNO_3 + H]^+$ by HRMALDITOFMS, and the complete ^1H, ^{13}C, DEPT-135, HMBC, and COSY data

sets are shown in Table 2. The 1D- and 2D-NMR data suggested high structural similarities with the aforementioned pyrrolizidine alkaloid (7*S*, 8*R*)-petranine (**1**), implying that compounds **1** and **2** are stereoisomers. As in compound **1**, characteristic signals for a 1,2-unsaturated pyrrolizidine alkaloid with a necic acid esterified at C-9 (angeloyl) and a bicyclic bridge structure between the oxygen at C-7 and the nitrogen atom of the base ring via a methylene group were evident (Table 2). In particular, compound **2** differed from (7*S*, 8*R*)-petranine (**1**) by more intense ROESY coupling between H-8 and H-7, and coupling between H_2-17 and H-3b, and between H-17a and H-5b (Fig. 1). Moreover, no ROSEY correlations were observed between H_2-17 and H-8; while it is recognized that the absence of a correlation is not definitive of structure, it is supportive of the molecular model of **2**. Thus, the relative configuration of position C-8 was proposed as (*S*). Due to sample limitation, we were unable to carry out X-ray crystallographic analysis to determine the absolute configuration of the two asymmetric centers. Despite considerable effort and due to paucity of sample, we were unable to obtain an IR spectrum of this compound as well. The name (7*S*, 8*S*)-petranine was ascribed to this new tricyclic pyrrolizidine alkaloid.

Table 2.

^{1}H-, ^{13}C-, DEPT-135, HMBC, and COSY NMR data (in $CDCl_3$) for compound **2**

Position	δ_C	**DEPT**	δ_H, **multiplicity (*J* in Hz)**	**HMBC (H → C)**	**COSY (H → H)**
1	134.6	C			
2	121.3	CH	5.83 bs		3a, 3b, 8, 9
3a	71.2	CH_2	4.54 d (15.5)		2, 3b, 8, 9
3b			4.78 d (15.5)		2, 3a, 9
5a	63.6	CH_2	4.09 m		5b, 6a, 6b
5b			4.35 m		5a, 6a, 6b
6a	34.8	CH_2	2.51 m		6b, 5a, 5b, 7
6b			2.69 m		6a, 5a, 5b, 7
7	70.6	CH	4.94 bs		6a, 6b, 8
8	88.1	CH	5.38 bs		2, 3a, 7, 9
9	59.9	CH_2	4.88 bs	1, 2, 11	2, 8, 3a
11	167.0	C			
12	127.0	C			
13	140.9	CH	6.20 qq (1.5, 7.5)		14, 15
14	16.3	CH_3	2.02 dq (1.3, 7.3)	12, 13	13
15	20.8	CH_3	1.9 d (1.5)	11, 12, 13	13
17a	69.1	CH_2	5.71 d (9.5)		17b
17b			5.39 d (9.5)		17a

Compound (**3**) (30 mg) was obtained as a white solid, and the molecular formula was determined as $[C_{14}H_{20}ClNO_3 + H]^+$ by HRMALDITOFMS (*m/z* 286.11942). Due to the unfortunate loss of the sample while preparing material for further NMR analysis, the only data sets obtained for this compound were ^1H and COSY, as shown in Table 3. The available NMR data suggested structural similarities with the aforementioned tricyclic pyrrolizidine alkaloids **1** and **2**. But due to lack of ROESY data, we could not deduce the relative configuration of C-7 and C-8. Thus, compound **3** could be either **3a** where C-7 and C-8 are both (*R*) or **3b** where C-7 and C-8 are (*R*) and (*S*), respectively. Fig. 2 describes a proposed biosynthetic scheme for this new class of tricyclic pyrrolizidine alkaloids.

Table 3.

^1H, COSY NMR data (in $CDCl_3$) for compound **3**

Position	**δ_H, multiplicity (*J* in Hz)**	**COSY (H → H)**
1		
2	5.81 bs	3b, 9
3a	4.52 d (16.5)	8, 9, 3b
3b	4.74 d (16.5)	2, 3a
5a	4.12 m	5b, 6a
5b	4.30 m	5a, 6b
6a	2.48 m	5a, 6b
6b	2.68 m	7, 5b, 6a
7	4.92 bs	8, 6b
8	5.47 bs	7, 3a
9	4.85 bs	2, 3a
11		
12		
13	6.18 q (7)	14, 15
14	2.00 dm (7)	13
15	1.92 s	13
17a	5.30 d (9.8)	17b
17b	5.67 d (9.8)	17a

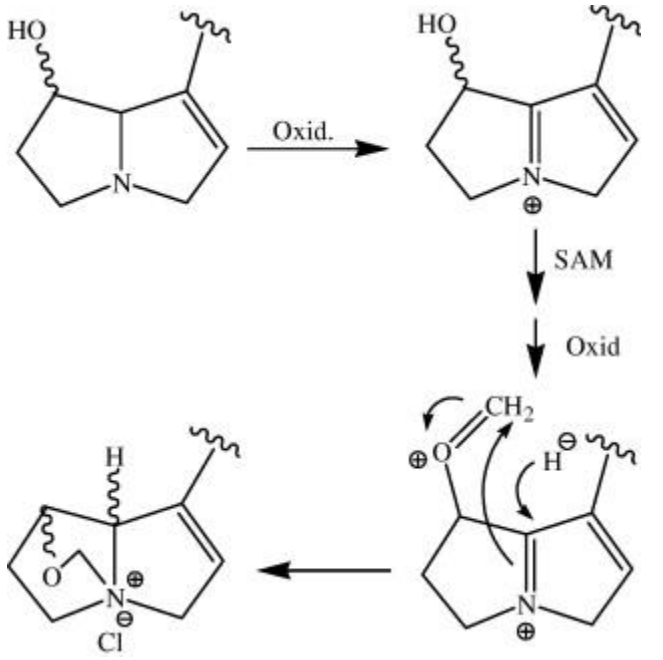


Fig. 2. Proposed biosynthetic scheme for the formation of compounds **1**, **2**, and **3**.

Two known pyrrolizidine alkaloids, but new to the species, were also isolated from fraction B, and their structures were identified by 1- and 2D-NMR, mass spectra analyses and comparisons to literature data. The spectral data for compound **4** (26 mg, 0.0004% w/w of whole plant) and compound **5** (54 mg, 0.0009% w/w of whole plant) were found to be in full agreement with those reported in the literature for 7-angeloylretronecine (Roeder et al., 1984) and 9-angeloylretronecine (Roitman, 1988), respectively.

We were curious if the tricyclic core of the new compounds imparted any anticancer activity. Thus, compounds **1**–**5** were tested for anticancer activity against a human cancer cell panel (MCF-7 human breast carcinoma, NCI-H460 human large cell lung carcinoma, and SF-268 human astrocytoma); no activity was observed (EC_{50} values > 20 μg/ml). During the screening process, activity was noted in the entire fraction B in the BST, and this may be due to other compounds that were not isolated in the course of this project.

3. Experimental

3.1. General experimental procedures

Optical rotation, IR, and UV data were obtained on a Rudolph Research Autopol® III polarimeter, a Nicolet Avatar 360 FT-IR, and a Cary 3 UV–vis spectrophotometer or a MultiSpec-1501, SHIMADZU® photodiode diode array spectrophotometer, respectively. Melting points were determined on a Bristoline melting point apparatus and are uncorrected. All NMR experiments were performed in $CDCl_3$ with TMS as an internal standard; gs-COSY, ROESY, gs-HSQC, gs-HMBC, ^{1}H NMR, ^{13}C NMR, and DEPT-135 spectra were acquired using a Varian Unity Inova-500 instrument with a 5 mm broad-band inverse probe with *z*-gradient (Varian Inc., Palo Alto, CA) and a Bruker 400 MHz NMR spectrometer (Industriestr., Fällanden, Switzerland). Low-resolution ESIMS and APCIMS were determined on an Agilent® (Palo Alto, CA, USA) ion-trap LC/MS system and on an Applied Biosystems/MDS Sciex API 150 EX single quadrupole LC/MS system (Applied Biosystems, Foster City, CA). High-resolutions MALDI-TOF-MS were acquired using an Applied Biosystems (Framingham, MA) TOF/TOF mass spectrometer, equipped with a Nd:YAG laser operating at 355 nm and 200 Hz; the instrument was operated in the reflectron mode, and the matrix employed was 2,5-dihydroxybenzoic acid prepared at a concentration of 9 mg/ml in 70:30 (v:v) acetonitrile-0.1% trifluoroacetic acid. The molecular models of compounds **1** and **2** were generated using Spartan ‘04 Macintosh version 1.0.1 (Wavefunction Inc., Irvin, CA). Molecular mechanics were used to calculate equilibrium geometry at the ground state for both isomers using the MMFF force field. Selected internuclear distances were measured and

compared to observed ROESY correlations. The observed interactions labeled in the Figure correspond to internuclear distances of 2.5–2.8 Å. HPLC was performed on a Lachrom® MERCK-HITACHI (Tokyo, Japan), equipped with quaternary gradient L-7150 pump, L-7455 Diode-Array Detector, L-7200 auto-sampler, and D-7000 Interface. The preparative HPLC column was a Hibar® MERCK, pre-packed column RT 250-25, Lichrosorb® RP-18 (7 μm). PTLC was carried out on 20 × 20 cm plates with silica gel F_{254} (Merck KGaA, Germany). Column chromatography was carried out using silica gel 60 (0.06–0.2 mm; 70–230 mesh) and TLC utilized silica gel 60 with gypsum and pigment addition for UV visualization (both from Scharlau Chemie S.A., Barcelona, Spain). TLC spots were visualized by UV (VILBER LOURMAT, 4 W–254 nm Tube) or made visible by spraying the developed plates with Dragendorff's reagent.

3.2. Plant material

The entire plant (flowers, leaves, stems, and roots) of *E. glomeratum* were collected during the flowering stage in April 2004 at the northern part of Jordan from Al-Wistyya, Irbid (elev. 521.2 m, lat. 32°33′182″N, long. 35°46′996″E). The plant was identified by Prof. Jameel Al-Lahham, a plant taxonomist in the Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan. A voucher specimen (PHC no. 111) was deposited in the herbarium of the Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. The plant material was air-dried at RT (22–23 °C) and ground to powder using a RetschMühle mill (RETSCH GmbH, Haan, Germany). Powdered materials were maintained at RT and protected from light until required for extraction and analysis.

3.3. Extraction and isolation

The plant material (6.2 kg) was soaked in MeOH at room temperature for one day with intermittent shaking followed by filtration to separate the marc; this process was repeated several times for exhaustive extraction. The filtrates were combined and dried under reduced pressure to yield the MeOH extract (628.4 g). This extract was dissolved in 0.5 M HCl and then extracted with dichloromethane (fraction A). The acidic aqueous residue was made alkaline (pH 9) with 25% NH_4OH followed by extraction (3×) with CH_2Cl_2 (fraction B). Using 1 M NaOH, the pH of the aqueous basic solution was raised to pH 11 and then extracted with CH_2Cl_2 (fraction C). The basic aqueous solution was then made acidic (pH 2) with 2 M HCl followed by reduction of *N*-oxides using zinc powder (20 g), which was added gradually with stirring for 24 h. The acidic aqueous residue was then made alkaline (pH 9) with 25% NH_4OH followed by extraction with CH_2Cl_2 (fraction D). Using 1 M NaOH, the pH of the aqueous basic solution was raised to pH 11 and then extracted (3×) with CH_2Cl_2 (fraction E). All fractions were dried in vacuo. Fraction B [984.2 mg, 0.063% (w/w)] was subjected to chromatography over silica gel using a gradient of 100% hexane to 100% CH_2Cl_2 to 50% MeOH in CH_2Cl_2 to yield 114 fractions, which were combined into 15 pools based on TLC. Pure compounds were isolated via reverse phase HPLC from the alkaloid-rich pools (5–14) using a solvent system of H_2O and CH_3CN (both containing 0.1% formic acid) via a gradient that initiated at 80:20 and increased to 20:80 over sixty minutes with a 10 ml/min flow rate and monitoring at 220 nm. Each injection utilized between 75 and 150 mg of material dissolved in 2 ml of MeOH and mobile phase in a 1:1 ratio. The purities of the isolated compounds were checked by TLC developed with either CH_2Cl_2:MeOH [85:15] or CH_2Cl_2:MeOH:H_2O with NaOH (25%) [85:15:2]. Further purifications were carried out via PTLC developed with CH_2Cl_2:MeOH (85:15).

3.4. Human cancer cell panel

Compounds **1**–**5** were evaluated for cytotoxicity using a panel of unrelated human cancer cell lines (MCF-7 human breast carcinoma (Barbara A. Karmanos Cancer Center, Detroit, MI), NCI-H460 human large cell lung carcinoma (American Type Culture Collection, Manassas, VA), and SF-268 human astrocytoma (NCI Developmental Therapeutics Program, Frederick, MD) as described previously (Alali et al., 2005).

3.5. (7S, 8R)-Petranine (1)

White powder, (59.0 mg); m.p. 103–105 °C; $[\alpha]_D^{23} - 10°$ (*c* 0.0006, MeOH); UV (MeOH) λ_{max} (log ε) nm: 216 (3.66); IR ν_{max} cm^{-1}: 2962, 2929 (base peak), 2854, 1718, 1458, 1385, 1232, 1155, 1045; ESIMS (positive mode) *m*/*z* (rel. int.): 286.3 $[M + H]^+$ (100); HRMALDITOFMS *m*/*z* 286.11966 $[M + H]^+$(calcd for

$[C_{14}H_{20}ClNO_3 + H]^+$, 286.1204); for ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR data see Table 1.

3.6. (7S, 8S)-Petranine (2)

White solid, (35 mg, 0.00058%); $[\alpha]_D^{23} - 5^\circ$ (*c* 0.0006, MeOH); UV (MeOH) λ_{max} (log ε) nm: 219 (3.60); ESIMS (positive mode) *m/z* (rel. int.): 286.5 $[M + H]^+$ (100); HRMALDITOFMS *m/z* 286.12162 $[M + H]^+$ (calcd for $[C_{14}H_{20}ClNO_3 + H]^+$, 286.1204); for ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR data see Table 1.

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