

Alkaloids from *Glaucium aleppicum* Papaveraceae

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ABSTRACT

Five alkaloids were isolated from the methanolic extract of the whole plant material of *Glaucium aleppicum* Boiss. & Hausskn. (Papaveraceae) using bioactivity-directed fractionation as part of a search for anticancer leads from medicinal plants of Jordan flora. Of these two were protopine-type alkaloids [allocryptopine (**1**) and protopine (**2**)], two were aporphine-type alkaloids [(+)-corydine (**4**) and (+)-isocorydine (**5**)], and (+)-norchelidonine (**3**). Compounds **3-5** were new to the species. The chemical structures of the isolated compounds were elucidated using a set of spectroscopic and spectrometric techniques, principally: MS, 1D-NMR (¹H and ¹³C) and 2D-NMR (COSY and HMQC). Compounds **1-5** were tested for general cytotoxicity using BST. (+)-Norchelidonine was found to possess the highest activity with LC₅₀ value of 0.41 µg/mL and hence evaluated for cytotoxicity against a human cancer cell panel. (+)-Norchelidonine induced antiproliferative activity against human melanoma MV3 and colorectal SW480 cancer cell lines in a dose-dependent manner with IC₅₀ values of 50 and 80 µg/mL, respectively.

Keywords: (+)-Norchelidonine, Cytotoxicity, *Glaucium*, Alkaloids, Papaveraceae.

INTRODUCTION

As part of an ongoing project to explore medicinal plants of Jordan for anticancer leads⁽¹⁻⁵⁾, crude methanolic extracts of *Glaucium aleppicum* Boiss. & Hausskn. (Papaveraceae) displayed promising cytotoxicity on the brine shrimp test (BST) [LC₅₀ 29.4 µg/mL] and hence was selected for further bioactivity-directed fractionation study.

G. aleppicum, which is found flowering from March

to May, is a perennial herb from the Irano-Turanian phytogeography region; sparingly crisp-papillose, green-glaucous, 20-50 cm^(6,7). An infusion of aerial parts is used in traditional medicine in Jordan to treat cataract⁽⁸⁾. Only two compounds, protopine and allocryptopine, were reported previously from *G. aleppicum* and have been tested for their ability to inhibit the enzyme aldose reductase, using crude lenses of male rats⁽⁹⁾.

RESULTS AND DISCUSSION

The whole plant material (leaves, stems, roots, and seeds) were treated as outlined in the experimental section. Fractionation and purification of the alkaloid rich fraction (fraction B), which showed potent cytotoxic activity in the BST (LC₅₀ of 1.2 µg/mL) resulted in the

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isolation and identification of five compounds (**1-5**). Two protopine-type alkaloids: allocryptopine (**1**) and protopine (**2**), two aporphine-type alkaloids: (+)-corydine (**4**) and (+)-isocorydine (**5**), and (+)-norchelidonine (**3**), with the last three being new to the species. The chemical structures of the isolated compounds were determined by 1- and 2D-NMR, mass spectra analyses, comparison with available authentic standards and by comparison with literature.

Compounds **1** (210 mg, 0.04%) and **2** (302 mg, 0.05%) were isolated as amorphous white powders. The molecular formulas were determined as $C_{21}H_{23}NO_5$ and $C_{20}H_{19}NO_5$ by LRESIMS, m/z 370 $[M+1]^+$ and 354 $[M+1]^+$, respectively. The 1D-NMR data of **1** and **2** suggested high structural similarity. In particular, **2** differed from **1** by the absence of the two methoxy groups and the appearance of extra characteristic two sharp singlets at δ_H 5.94 and 5.92 corresponding to a methylenedioxy group. Although the parent molecular ion and the MS fragments of **1** were in good agreement with an authentic standard of allocryptopine, their proton NMR spectra were not, compound **1** showed few extra peaks. Careful examination of those peaks suggested that compound **1** was a mixture of two tautomers existing in equilibrium, the free base form (tricyclic) and the quaternary ion form (tetracyclic), as shown in Figure 2. The same phenomenon was also noticed for **2**. This tautomerization was facilitated in acidic medium; recall that formic acid was used during the purification. To test our hypothesis, we recrystallized the samples in basic ammonium solution, where the NMR spectra of **1** and **2** now match perfectly with those reported in the literature for allocryptopine and protopine, respectively⁽¹⁰⁾, both reported previously from *G. aleppicum*⁽⁹⁾. The phenomena of tautomerization of allocryptopine and protopine was reported recently by Kubala *et al.* and was confirmed by x-ray crystallography and NMR⁽¹¹⁾

Compound **3** (35 mg, 0.01% w/w) was isolated as an amorphous yellow powder. LRESIMS data showed a molecular ion peak of m/z 340 $[M+1]^+$, corresponding to the chemical formula $C_{19}H_{17}NO_5$. The spectral data of compound **3** were found in agreement with those reported

for (+)-norchelidonine, and this is the first report of this compound from *G. aleppicum*⁽¹²⁾. A specific rotation of $+74^\circ$ ($c = 0.07$, $CHCl_3$) was reported for this compound⁽¹²⁾.

Compound **4** (7 mg, 0.003%) and compound **5** (8 mg, 0.003%) were isolated as amorphous yellow powders. Both compounds showed similar LRESIMS spectra of m/z 342 $[M+1]^+$, corresponding to the molecular formula $C_{20}H_{23}NO_4$. The two compounds showed high similarities in their 1D-NMR data as well. The spectra of the two compounds **4** and **5** were found to be in agreement with those reported for (+)-corydine and (+)-isocorydine, respectively, and were new to the species⁽¹³⁾. Specific rotations values of $+266$ (c 0.36, $CHCl_3$) and $+235$ ($c = 1.5$, $CHCl_3$) were reported for (+)-corydine and (+)-isocorydine, respectively, in the literature⁽¹³⁾.

Compounds **1-5** were tested for general cytotoxicity against the BST. Compound **3** was the most toxic with an LC_{50} value of $0.41 \mu g/mL$. Compound **3** was further assayed for cytotoxicity. When tested against human melanoma MV3 and colorectal SW480 cell lines, it showed a dose-dependent moderate cytotoxicity, with IC_{50} values of 50 and $80 \mu g/mL$, respectively.

In conclusion, using bioactivity-directed fractionation methodology, five compounds were isolated and identified from *Glaucium aleppicum* Boiss. & Hausskn. (Papaveraceae), namely: allocryptopine, protopine, (+)-corydine, (+)-isocorydine, and (+)-norchelidonine, with the last three were new to the species. The cytotoxic evaluation of the isolated compounds using BST revealed that (+)-norchelidonine was the most potent with LC_{50} of $0.41 \mu g/mL$. When assayed for cytotoxicity against the two cancer cell lines, MV3 and SW480, (+)-norchelidonine showed moderate cytotoxic activity with IC_{50} values of 50 and $80 \mu g/mL$, respectively.

General Experimental

NMR experiments were performed in $CDCl_3$ with TMS as an internal standard; 1D- (1H and ^{13}C), 2D-NMR (gs-HMQC) spectra were run using a Bruker instrument. Low resolution APCIMS were determined on an Applied Biosystems/MDS Sciex API 200 EX triple quadrupole LC/MS system, while an Agilent 1100 series LC/MSD trap

was utilized for LRESIMS (both from Applied Biosystems, Foster City, CA). HPLC was performed on a Lachrom Merck-Hitachi (Tokyo, Japan), equipped with a quaternary gradient L-7150 pump, L-7455 diode-array detector, L-7200 autosampler, and D-7000 interface. The preparative HPLC column was a Hibar Merck prepacked column RT 250-25, Lichrosorb RP-18 (7 μ m). PTLC was carried out on 20 \times 20 cm plates with silica gel F₂₅₄ (Merck KGaA, Germany). Column chromatography was carried out using dextran gel (Sephadex[®] LH-20) (Sigma, Switzerland) and silica gel 60 (0.06-0.2 mm; 70-230 mesh) (Scharlau Chemie S.A., Barcelona, Spain). For TLC silica gel 60 with gypsum and pigment addition for UV-visualization was utilized (Scharlau Chemie S.A., Barcelona, Spain). TLC spots were visualized by UV (Vilber Lourmat, 4 W-254 nm tube). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Molecular Probes (Eugene, UR).

Plant material

The whole plant material of *G. aleppicum* (leaves, stems, roots, and seeds) were collected during the germinating stage in June 2009 in the northern part of Jordan from the campus of Jordan University of Science and Technology (JUST), Irbid, Jordan. A voucher specimen (PHC-115) was deposited in the herbarium of the Faculty of Pharmacy, JUST. The collected plant material was identified by Mohammad Gharaibeh, plant taxonomist, Faculty of Agriculture, JUST. The plant material was air-dried at room temperature (22-23 °C) and ground to powder using a RetschMühle mill (RETSCH GmbH, Haan, Germany). Powdered materials were maintained at room temperature and protected from light until required for extraction and analysis.

Plant's samples preparation and analysis

The dried plant material was extracted using the method described by Shafiee et al 1979, with modifications⁽¹⁴⁾. In brief, about 1 kg of dried plant material was extracted with *n*-hexane using Soxhlet apparatus, followed by exhaustive extraction with MeOH to yield crude MeOH extract (93 g). The extract was then dissolved in 50% acetic acid and extracted three times with dichloromethane (fraction A). The aqueous acid

residue was made alkaline (pH 9) with 15% NH₄OH followed by extraction four times with dichloromethane (fraction B). The aqueous fraction was designated fraction C. The fractions (A-C) were dried under vacuum and then about 5 g of fraction B (the most active on BST) was fractionated over dextran gel column (Sephadex[®] LH-20) using 100% ethanol. Similar fractions were combined into 7 pools based on their TLC using the solvent system: dichloromethane:methanol:ammonia (88:10:2). Pool 3 (1.9 g) showed higher concentration of alkaloids as evidenced from spraying the TLC plates with Dragendorff reagent⁽¹⁵⁾, and hence subjected to chromatography over silica gel column using a gradient of 100% *n*-hexane to 100% CH₂Cl₂ to 20% MeOH in CH₂Cl₂. Similar fractions were combined into 5 pools based on their TLC using 3 different solvent systems: A, ethyl acetate: methanol: ammonia (83:15:2); B, dichloromethane: methanol: ammonia (88:10:2) and C, dichloromethane: methanol: ammonia (93:5:2). The presence of alkaloids was checked by spraying with Dragendorff reagent⁽¹⁵⁾. Pure compounds were isolated from the alkaloid-rich pools via semipreparative HPLC using a gradient solvent system of MeCN or MeOH and H₂O buffered with 40 mM ammonium acetate, pH adjusted into 3.5 using formic acid (10:90 to 95:05 over 50 min, total run time 90 min), with a 8 mL flow rate, monitoring at 254 nm, and injecting 100 mg of material dissolved in 2 mL of MeOH and mobile phase in a 1:1 ratio. Further purifications were carried out using PTLC developed in either of the mobile phases mentioned above. The chemical structures of the isolated compounds were elucidated using a set of spectroscopic and spectrometric techniques, principally: MS, 1D-NMR (¹H and ¹³C) and 2D-NMR (COSY and HMQC) and by comparison with available authentic standards.

Brine shrimp lethality test (BST)

The BST was used to test the crude methanolic extract, fractions A-C and the pure compounds for cytotoxic activity. It was performed as described previously^(16,17).

Cytotoxicity assay

Human melanoma MV3 and colorectal SW480 cell

lines were generously provided by Dr. Rick F. Thorne (University of Newcastle, Australia) and were cultured in DMEM containing 10% FCS (Bio Whittaker, Verviers, Belgium). The cytotoxicity measurements was determined using MTT assays as described previously⁽¹⁸⁾. Briefly, cells were seeded at 5000/well onto flat-bottomed 96-well culture plates and allowed to grow for

24 h before the desired treatment. Cells were then labeled with MTT from the Vybrant MTT Cell Proliferation Assay Kit (Molecular Probes, Eugene, UR) according to the manufacturer's instruction and resulting formazan was solubilized with DMSO. Absorbance was read in a microplate reader at 540 nm.

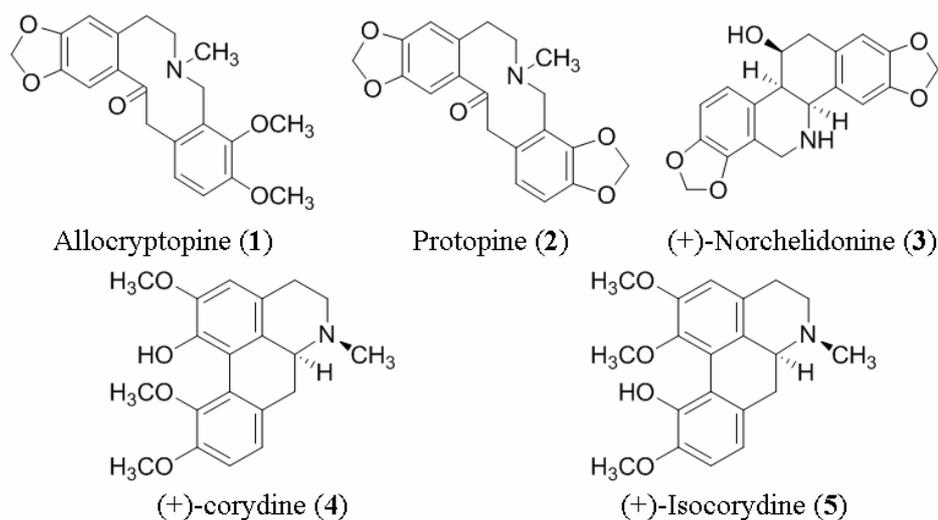


Figure 1: Chemical structures of isolated compounds.

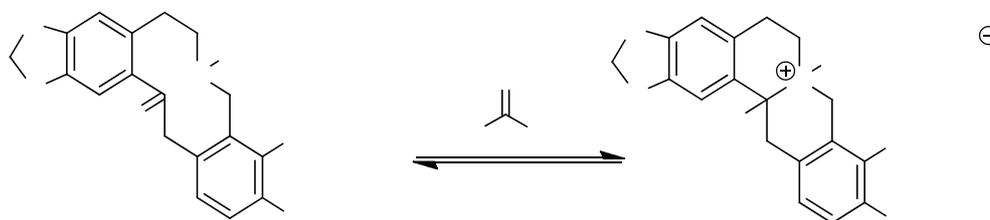


Figure 2 : Tautomerization of allocryptopine in acidic media.

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	<i>1</i>	<i>3</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>1</i>	
.	22110	3030 . .					1
	22110	3030 . .					2
	22110	3030 . .					3

Glaucium aleppicum

alocryptopine (1), protopine (2), (+)-norchelidonine (3), (+)-corydine (4) and (+)-isocorydine (5)

(+)-

0.41 (LC₅₀)

Norchelidonine

80 50 (IC₅₀)

. Papaveraceae

Glaucium

(+)-Norchelidonine :

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2013/6/30