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Bioactive isoquinoline alkaloids from *Glaucium arabicum*

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ABSTRACT

Phytochemical investigation of the aerial parts of *Glaucium arabicum* Fresen. (Papaveraceae) led to the isolation of two previously undescribed isoquinoline alkaloids araglaucine A, and araglaucine B, together with seven known ones 1-[(3`,4`-dimethoxy-2`-methylcarboxy)benzoyl]-6,7-methylenedioxy isoquinoline (araglaucine C), (7*R*,14*S*)-*trans*-*N*-methylcanadinium nitrate, (*R*,*S*)-*trans*-*N*-methylstylopine, 14-hydroxy-*N*-methyl canadine, 14-hydroxy-*N*-methyl stylopine, protopine, norsanguinarine, as well as *β*-sitosterol, and *β*-sitosterol 3-*O*-*β*–D-glucoside. Their structural elucidation was based on the measurements of 1D, 2D NMR, HRESIMS, UV, IR and X-ray crystallography. The compounds were evaluated for their anti-melanogenesis activity using B16 melanoma cell lines. Compound (7*R*,14*S*)-*trans*-*N*-methylcanadinium nitrate exhibited a promising melanin synthesis inhibitory activity (~35%) at concentration 5 μg/ml (12.01 μM) with low cytotoxicity (~12%).

1. Introduction

Genus *Glaucium* Mill. (Papaveraceae) includes about 23 species distributed in Europe, Mediterranean region, southwest and central Asia. It is represented in Egypt by four species; *G. corniculatum* L., *G. flavum* Cranz., G. *grandiflorum* Boiss., and *G. arabicum* Fresen. (Boulos, 2009; Täckholm, 1974). *Glaucium arabicum* Fresen. (Papaveraceae) is a wild herb endemic to Sinai Peninsula where it is locally known as No'maan or Ne'man. It grows wildly in Palestine, Jordan, Iraq and Libya as well (Heywood, 1978). The species of *Glaucium* have been used in Iranian herbal medicine as laxative, antidiabetic, hypnotic, antifungal and for treatment of dermatitis (Morteza-Semnani et al., 2003). *Glaucium arabicum* is used in the folk medicine of the Bedouins living in Sinai for the management of eye and skin infections (Khafagi and Dewedar, 2000). Plants belonging to the genus *Glaucium* are chemically characterized by their alkaloidal content especially isoquinoline alkaloids. Many of the isolated alkaloids exhibited versatile biological activities such as antitussive, antimicrobial, antispasmodic, anti-histaminic, anti-inflammatory, cytotoxic, anti-platelet aggregation activities and in the treatment of intestinal disorders (Shiomoto et al., 1991; Chia et al., 2006; Grycová et al., 2007).

Hyperpigmentation is a common harmless skin condition in which melanocytes are stimulated by sunlight exposure, inflammation, free radicals and hormonal changes to overproduce melanin. Therefore, seeking new natural compounds exhibiting melanin synthesis inhibitory activity is the aim of many researches.

Although plants of genus *Glaucium* were extensively studied for their alkaloid content and biological activities as antimicrobial and smooth muscle relaxant activities, there is a lack of knowledge about the alkaloids of the aerial parts of *G. arabicum* growing in Sinai Peninsula and their antimelanogenesis activity. Therefore, the aim of this work was to study the isolation & structural elucidation of the alkaloids of the aerial parts of *G. arabicum* growing in Sinai. Additionally, the biological evaluation of the isolated compounds regarding their melanin synthesis inhibitory activity was studied.

2. Results and discussion

2.1. Identification of the isolated compounds

Using a combination of chromatographic techniques, eleven compounds (**1** - **11**) were isolated from the methylene chloride extract of the alkalinized aerial parts of *G. arabicum*. Their structural elucidations were performed using extensive physicochemical and spectroscopic methods including 1D-, 2D-NMR, HRESI$^+$MS, UV, IR, and X-ray crystallographic measurements. Compounds **1** and **2** are new isoquinoline

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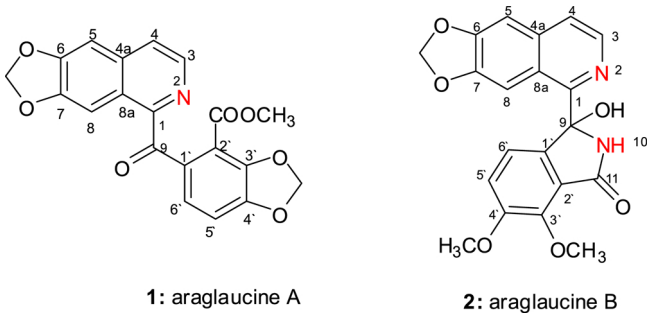

1: araglaucine A **2:** araglaucine B

Fig. 1. Structures of compounds **1 and 2.**

derivatives; compounds **3**, **6**, and **7** are reported in this study from the family Papaveraceae for the first time; compounds **5** and **9–11** are isolated from *G. arabicum* for the first time.

Compound **1** (Fig. 1) was obtained as a yellow powder with a molecular formula $C_{20}H_{13}NO_7$ on the basis of accurate mass measurement (HRESI$^+$MS, *m/z* 380.0756 $[M+H]^+$, Figure S6) and the number of signals in ^{1}H, ^{13}C NMR and HSQC spectra (Figures S1-S3). Compound **1** is composed of isoquinoline moiety connected via a carbonyl bridge to a substituted phenyl group. The ^{1}H and ^{13}C NMR spectra showed one methyl, two methylenes, six aromatic methines, four oxygenated aromatic quaternary carbons, two carbonyls and five quaternary carbons. The ^{1}H NMR spectrum exhibited two characteristic methylene protons OCH_2O/C-6,7 and OCH_2O/C-3`,4` at $\delta_{H/C}$ 6.15/101.9 and 6.14/102.8, respectively. Their high down field shift in the ^{1}H NMR and ^{13}C NMR spectra indicating their attachment to two oxygen atoms producing methylenedioxy groups (IR 1034 cm^{-1}). OCH_2O/C-6,7 is connected to the aromatic C-6 and C-7 via oxygen as indicated by HMBC correlations from OCH_2O/C-6,7 (δ_H 6.15) to C-6 and C-7 (δ_C 150.9 and 150.1, respectively) (Fig. 2, Figure S5). The ^{1}H NMR spectrum showed also two singlet aromatic protons H-5 and H-8 and they have HMBC correlations with both C-6 and C-7. Also H-5 and H-8 having HMBC correlations to the *sp*^{2} carbons C-4a and C-8a. The ^{1}H NMR spectrum showed also characteristic aromatic *ortho* coupled protons at δ_H 8.31 (d, J = 5.2) and δ_H 7.57 (d, J = 5.2) for H-3 and H-4, respectively, which were confirmed by COSY correlations between them (Figure S4). H-3 has HMBC correlation to the resonance peak at δ_C 152.3 for C-1 through an N atom due to the chemical shifts of H-3 and C-1 at $\delta_{H/C}$ 8.31/140.2 and δ_C 152.3, respectively. The presence of N is confirmed by the odd mass number at 380.0756 $[M+H]^+$ and by measuring the ^{1}H ^{15}N HMBC (Figure S7) which showed a correlation at 57.3 ppm from both H-3 and H-4 to the N atom. The coupling constant J = 5.2 together with UV absorption maxima at 241 and 334 nm are characteristic for an isoquinoline alkaloid (Rahman et al., 1992, 1995; Kim et al., 2010). The aforementioned partial structure of compound **1** was identified as a substituted isoquinoline.

The second methylenedioxy protons OCH_2O/C-3`,4`at $\delta_{H/C}$ 6.14/102.8 have HMBC correlations to the quaternary aromatic carbons C-3` and C-4`. The ^{1}H NMR spectrum also showed *ortho* coupled aromatic methine signals at $\delta_{H/C}$ 6.97/110.0 and 7.27/125.6 for CH-5` and CH-6`, respectively, which was confirmed by COSY correlations (Figure S4). The H-5` and H-6` showed HMBC correlations to the quaternary aromatic carbons C-1`/C-3` and C-2`/C-4`, respectively indicating the presence of tetra-substituted aromatic moiety.

The ^{1}H and ^{13}C NMR spectra showed the presence of a methyl ester at $\delta_{H/C}$ 3.34/52.0 and a carbonyl moiety at δ_C 164.7/CO representing $COOCH_3$/C-2` (IR 1700 cm$^{-1}$) confirmed by HMBC correlation between $\delta_H$ 3.34 and CO. The methyl ester $COOCH_3$ substitutes the aromatic moiety at C-2` due to HMBC correlation between H-6` and $COOCH_3$. The $^{13}$C-NMR spectrum showed a resonance peak at $\delta_C$ 195.6 (C-9) which is characteristic for a carbonyl group (IR 1679 cm$^{-1}$). This carbonyl group is connected to the aromatic moiety at carbon C-1` as shown by its HMBC correlation with δ_H 7.27 (H-6`). The carbonyl group has an *sp2* carbon, attached to the aromatic moiety at carbon C-1` and to the isoquinoline unit at C-1 and this carbonyl position was in agreement with the similar known derivative compound **3** (Min et al., 2006).

From the above results, compound **1** was identified as 1-(3`, 4`-methylenedioxy-2`-methylcarboxybenzoyl)-6,7-methylenedioxyisoquinoline.

Compound **2** has a molecular formula of $C_{20}H_{16}N_2O_6$ which was determined from the $[M+H\text{-}H_2O]^+$ peak at *m/z* 363.0977 in the HRESI$^+$MS (Figure S12) and the number of protons and carbons in the 1D spectra (Figures S8 & S9). Compound **2** is unprecedented one since it is composed of both isoquinoline and iso-indole moieties. The isoquinoline moiety of compound **2** has almost the same spectroscopic data as that of compound **1**. Additionally compound **2** has an iso-indol-3`-one moiety attached directly to the isoquinoline moiety, which was confirmed as follows. The $^1$H NMR spectrum showed two resonance peaks at $\delta_H$ 4.19 and $\delta_H$ 3.87 for two methoxy protons and they are attached to the aromatic quaternary carbons C-3` and C-4`, respectively due to the HMBC correlations to the respective aromatic carbons. The $^1$H NMR spectrum was characterized also by the resonance peaks of the *ortho* coupled protons H-5` and H-6` ($J$ = 8.2). H-5` and H-6` having HMBC correlations (Figures 2 & S11) to C-1`, C-3` and C-4`, C-2`, respectively creating the aromatic ring of the indole nucleus. The ^{13}C NMR spectrum showed a resonance peak for the amide carbonyl group CO-11 at δc 168.2 and the ^{1}H NMR spectrum showed a resonance peak for NH at δ_H 6.28. The amide carbonyl CO-11 is of lactam type based on the chemical shift of C-11 at 168.2 ppm (Elsebai et al., 2011a and Elsebai et al., 2011b, 2012. The lactam functionality was further

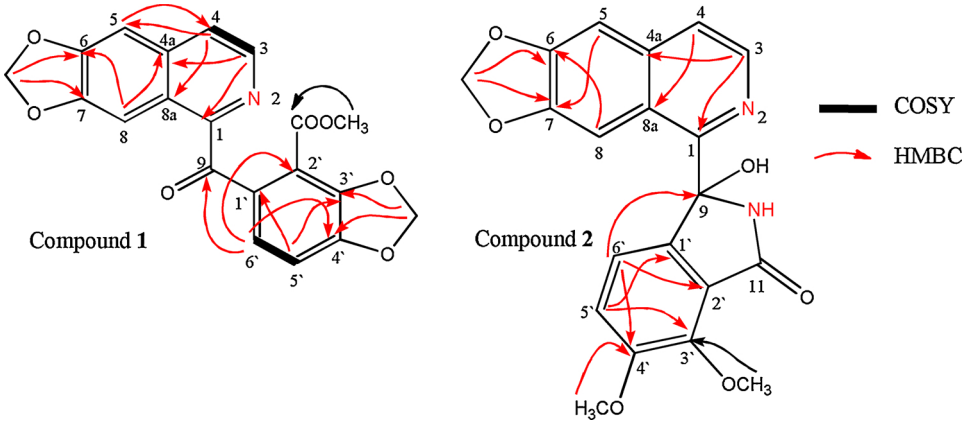

Fig. 2. Key COSY and HMBC correlations for compounds **1** and **2.**