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NAPHTHAQUINONES OF *ALKANNA ORIENTALIS* (L.) BOISS

Wafaa A. Tawfik, *Khaled A. Shams, Nahla S. Abdel-Azim, Nahed M. Hassan and Shams I. Ismail

Department of Phytochemistry., National Research Centre, 12311, Dokki, Egypt
Pharmacognosy and Chemistry of Medicinal Plants Dep., National Research Centre, 12311, Dokki, Egypt
***E-mail:** khaledashams@hotmail.com, **Fax:** 002 02 3370 931

Abstract

The roots of *Alkanna orientalis* (L.) Boiss yielded α- methyl-n-butyl alkannin **(compound 1)** and alkannin acetate **(compound 2)**. The compounds were identified by UV, MS, ^{1}H NMR and ^{13}C NMR. Quantitative determination of α- methyl-n-butyl alkannin and alkannin acetate in *Alkanna orientalis* (L) Boiss roots was established by TLC densitometry.

Keywords: ***Alkanna orientalis***, naphthaquinones, NMR, α- methyl-n-butyl alkannin, alkannin acetate, TLC densitometry.

Introduction

The majority of naphthalene derivatives present in nature are quinones and are usually found in the createnchyma of the roots of about 150 plant species mostly belonging to the family Boraginaceae. Now, it has been proved beyond doubt that not only red coloration of the roots of the boraginaceous taxa but also their therapeutic actions are due to the presence of naphthaquinones – isohexenylnaphthazarins. A number of cosmetics, dyes, pharmaceutical preparations and food colorants have also been prepared from time to time either from the isohexenylnaphthazarin pigments and their derivatives or directly from the crude plant extract having these naphthaquinones (Futagoishi and Abe, 1972; Hatinguais and Belle et al 1980; Tabata and Hond, 1988). Many workers throughout the world have isolated and identified these compounds from the species of the genera Alkanna, Anchusa, Arnebia, Echium, Lithospermum and Onosma. No data were found on the naphthaquinones of *Alkanna orientalis* growing in St. Katherine, Egypt.
So, the aim of this work was to isolate and identify the naphthaquinone derivatives from the roots of *Alkanna orientalis* (L.) Boiss. Also the quantitative determination of the isolated compounds was established by TLC-densitometry.

Experimental

General

UV absorbance were measured using Schimadzu UV 2401 PC UV- recording spectrophotometer.EI-MS was done using Finnigan MATSSQ 700 system. FAB-MS on VG 70 SEDQ instrument. NMR spectra were measured using Broker DRX-400 at 400 MHz for ^{1}H and at 100MHz for ^{13}C-NMR. Analytical grade solvents and reagents (Merck) were used.
Thin-layer chromatography was performed on Merck precoated Silica gel F 254 Plates while column chromatography was carried out using Merck silica gel 60 (200-250 mesh) as adsorbent. Solvent systems for

TLC was 15% EtOAc in-hexane. The mobile phase for TLC- densitometric analysis was n-hexane- EtOAc-AcOH (100:15:1).

Plant Material

Alkanna orientalis (L.) Boiss roots (s.n.Cairo University Herbarium) were collected from St. Katherine, South Sinai in October 1999, and authenticated by Prof. S. F. Khalifa, Botany Dept,. Faculty of Science, Ain Shams University.

Extraction and Isolation

The dried powdered roots (1kg) were soxhlet extracted with n-hexane for 2h and was evaporated to dryness. The residue was dissolved in methanol and left overnight in the refrigerator to separate the soluble fraction (4g). The methanol soluble fraction was subjected to column chromatography (CC) on silica gel 60 using n-hexane with increasing amounts of benzene (4%, 8%, 15%, 30%, 50%, 75% and 100%) and finally eluted with benzene-EtOAc (75 : 25). Fractions were pooled after monitoring with TLC.Fraction 4 (0.5g eluted with hexane – benzene, 85: 15) was further purified by CC on silica gel 60 and the elution was initiated with hexane – EtOAc, 2%. The quinone containing – fractions (8 – 14) were pooled and rechromatographed on silica gel 60 column and eluted with 1% EtOAc in hexane to give 30 mg of a single compound **(compound 1)** with R_f 0.3 which appeared as dark violet spot in day light and brike red in the UV-light (254-366nm). Also, fraction 5 which was eluted with hexane – benzene (70 : 30) was subjected to CC on silica gel 60 and eluted with 5% EtOAc in hexane. The quinone containing fractions (4 – 12) were pooled and rechromatographed on silica gel 60 column and eluted with 2.5% EtOAc in hexane to give **compound 2** with R_f 0.42 which appeared as faint violet spot in the day light and red in the UV-light(254-366nm).

Sample Solutions

Sample solutions were prepared by dissolving 30 mg of extracted samples in 30 ml EtOAc.
Standard stock solutions were prepared by dissolving 50 mg of the isolated pure compounds in 50 ml EtOAc.

TLC- densitometric assay

A CAMAG automatic TLC Scanner with CATS evaluation software was used with the following settings: wavelength 520 nm, scanning speed 20 mm/s, multi level calibration via peak area by polynomial regression, silica gel 60 F254 (20 X 20 cm, Merck) plates were used. Samples were applied band wise using CAMAG automatic TLC sampler III under the following conditions: distance from lower edge = 20 mm, band length = 8 mm, track distance = 14, No. of applications = 12.

Results and Discussion

α- Methyl-n-butyl alkannin (1)

The UV absorption spectrum of **1** in n-hexane showed peaks at λ_{max} 275, 472, 523 and 565 nm which are characteristic of naphthaquinone – type structure (Papageorgiou, 1977).
The ^{1}H-NMR ($CDCl_3$) showed signals at δ 7.16 (s, s, 2H, H-6, H-7) and at 6.96 (s, 1H, H-2). Two singlet protons appeared at δ 12.57 and 12.39 ppm for the two peri-hydroxyl groups (Papageorgiou, 1979). Two protons (H-1` and H-3`) were coupled with the methylene group protons at δ 2.6 (dd, 1H, J= 3.9 and 7.63 Hz, H-1`) and δ 2.43 (t, 1H, J=6.66 Hz, H-3`). Another important peaks at δ 1.66 (s, 3H, H-5`) and 1.56 (s, 3H, H-6`) were detected. These data suggested the presence of an ester group of shikonin (alkannin) at position 1`.
Two signals at δ 0.89 (t, J= 7.45 Hz, H-4``) and 1.20 (d, J= 7.0 Hz, H-5``) were attributed to the methyl protons at C-4`` and C-5``. Also methylene group protons at δ 1.7 and 1.5 appeared (m, 2H-3``) and the signal at δ 2.41 was attributed to one proton on C-2``(1H-2``). So the nature of the side chain was shown to be α-methyl-n-butyl.

The ^{13}C-NMR spectrum showed the most important signals like C-4`` at 11.5 ppm and C-5`` at 17.9 ppm characteristic of two methyl groups. The signal for C-3`` of the compound appeared at 26.6 ppm and for C-2`` at 41.2 ppm. The carbonyl carbon appeared at 171.8 ppm.

The HMBC experiment supported the structure of the side chain in which among others connectivity correlations between the carbonyl carbon at 171.80 ppm and the protons at δ 1.20 ppm (δc 17.9, C-5``), 1.5 ppm (δc 26.6, C-3``) and 2.43 ppm (δc 41.2, C-2``).

EI-MS of **1** showed a molecular ion peak at m/z 372 which corresponds to the molecular formula C_{21} H_{24} O_6 . Another important fragments at m/z 270 (M^+ - α- methyl butyric acid) and 255 (M^+ - α- methyl butyric acid - $^+CH_3$). This fragmentation pattern confirm the presence of α- methyl butyric acid as a side chain and was similar to those reported for alkannin (Shukla, et al., 1969). So, from the spectroscopic data **1** was identified as α- methyl-n-butyl alkannin.

Alkannin acetate (2)

The UV absorption spectrum of **2** in hexane showed peaks at λ_{max} 275, 472, 523 and 565 nm which are characteristic of the naphthaquinone skeleton (Papageorgiou, 1977).

The ^{1}H-NMR spectrum of **2** ($CDCl_3$) showed signals identical to those of **1**. The two singlet protons at δ 7.15 ppm (S, 2H, H-6, H-7), 6.95 (S, 1H, H-3`), 12.3 and 12.5 of the two peri-hydroxyl groups, 6.01 (dd, 1H, J= 4.03, 7.2 Hz), 5.15 (t, 1H, J= 7.3 Hz), two methyl protons at δ 1.58 and 1.69 ppm, 2.49 and 2.55 (m, 2H, H-2`). Thus, **2** is a naphthaquinone derivative which has a structure similar to **1** but differ in the side chain.

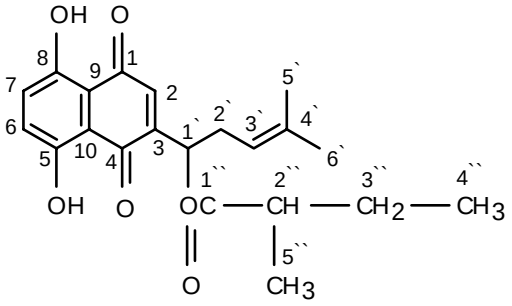
The ester group at position 1``was identified as acetic group due to the presence of only one singlet signal of three protons at δ 2.10 ppm.

EI-MS of **2** showed a molecular ion peak at m/z 329 corresponding to the molecular formula C_{18} H_{18} O_6. The fragment at m/z 270 (M^+ - 60) confirmed the presence of an acetic group as a side chain. Another important fragment m/z 235 was actually (M^+ - isohexyl group + $^+CH_3$). The presence of an ion peak at m/z 189 confirmed the presence of an isohexyl group and acetic acid as a side chain. Therefore, **2** was identified as Alkannin acetate.

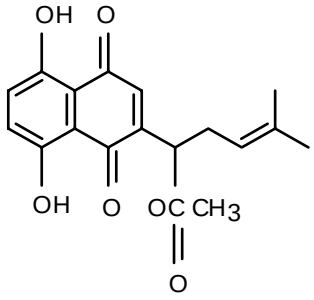
Naphthaquinones in *Alkanna orientalis* (L) Boiss roots were quantitatively determined by TLC-desitometry. The spots with R_f values of approximately 0.73 and 0.56 (n-hexane,ethyl acetate, acetic acid; 100:15:1) were α- methyl-n-butyl alkannin **(1)** and Alkannin acetate **(2).** The spot areas of each compound were found to range linearly against measured concentrations.

Compounds **1** and **2**, in the n-hexane extract of the root were found to be 21.98 and 27.99 %, respectively.

Results obtained in this study showed that the TLC-densitometric method can be used in the quantitative evaluation of naphthaquinone derivatives existing in plant extracts for its simplicity, rapidity and reduced cost.



α-methyl-n-butyl alkannin **(1)**



Alkannin acetate **(2)**

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