On the Constituents of the Wild Egyptian Plant *Onopordon alexandrinum* Boiss.

G. M. Wassel

Pharmaceutical Sciences Laboratory, National Research Centre Dokki, Cairo

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The study of the nitrogenous bases of *Onopordon alexandrinum* Boiss. resulted on the isolation of stachydrine and choline. The flavonoid components detected are: Luteolin-7-monoglucoside, apigenin-7-glucoside and quercetin.

Introduction

Onopordon alexandrinum Boiss. (Compositae) has been highly reputed as a curing and useful plant. It is a wild desert plant growing widely [1] in the Egyptian Mediterranean coastal strip from Sallum to Rafah. The aerial parts are used by the native as expectorant, for healing wounds and for the treatment of leprosy.

The occurrence of alkaloids and/or nitrogenous bases as well as flavonoids has been established from the preliminary screening.

Since no detailed study of these compounds has been published, the following investigation is reported.

Experimental

Material

The aerial parts of *Onopordon alexandrinum* Boiss. were collected from Burg-El-Arab in April. The plant was kindly authenticated by Prof. V. Täckholm at the Herbarium of the Faculty of Science Cairo University.

Adsorbent

Silica gel G. was used as well as the solvent systems cited in the text, according to the studied group of compound.

A. Study of the Nitrogenous bases

200 g of the defatted powdered plants were extracted with 70% methanol, the hydro-methanolic extract was reduced under vacuo and extracted with 2% hydrochloric acid, till the aqueous acidic layer gave a negative test for alkaloids. The aqueous acidic layer was shaken with benzene to remove any extraneous matter and then neutralized with ammonium hydroxide to pH 7.

This alkaline extract was adjusted to pH 4 with 10% hydrochloric acid then warmed on a water bath for 15 min. The bases were then precipitated with a 4% aqueous ammonium reineckate solution following the modified procedure of Panous [2]. The solution was allowed to stand at 0 °C for 24 hours, and then filtered. The precipitate was dissolved in methanol and the methanolic solution was passed through a column of anion exchange resin Amberlite IRA 400. The methanol eluate was evaporated under reduced pressure and applied on TLC (silicagel G.) using methanol-water (50 + 50) and Dragendorff as locating reagent. An orange spot R_F 0.48 (A) and a violet one R_F 0.11 (B) were located.

The substances corresponding to the above separated spots were isolated by preparative TLC (1 mm thick) using the same conditions. After developing the zones containing the nitrogenous bases were scrapped and eluted separately with 50% methanol.

The methanolic solution of substance A (R_F 0.48) was evaporated under reduced pressure yielding a white residue. This residue, after repeated crystallisation from absolute ethanol yielded transparent colourless crystals m. p. 235 °C with decomposition. Its hydrochloride melted at 221 – 222 °C. The structure of the compound and its identity to stachydrine was proved by TLC, mixed m. p. and IR spectra with authentic stachydrine.

The methanolic solution of substance B (R_F 0.11) upon evaporation to dryness yielded a viscous oily residue. Its reineckate salt was prepared, gave a m. p. of 284 – 286 °C with decomposition undepressed upon mixing with authenic choline reineckate and showed identical IR spectra.

B. Flavonoids

The defatted powdered drugs (250 g) were extracted with ethyl acetate. TLC of the ethyl acetate

Requests for reprints should be sent to Prof. Dr. G. M. Wassel, Pharmaceutical Sciences Laboratory National Research Centre, *Dokki-Cairo*, Egypt.

extract using different solvent systems, *viz.* I-Chloroform-methanol-formamide (80 + 19 + 1), and II-ethyl acetate-benzene-methanol-formamide (80 + 40 + 19 + 1), revealed the presence of 3 flavonoid components. They possess the same R_F as luteolin-7-monoglucoside, apigenin-7-glucoside and quercetin by cochromatography with authentic substances.

Detection of the flavonoids was carried out by exposing the plates to UV light before and after exposing to ammonia vapour or spraying with aluminium chloride reagent [6]. The isolation of the flavone glucosides and quercetin was carried out using thick layer chromatoplates and the zones corresponding to that of luteolin-7-monoglucoside, apigenin-7-glucoside and quercetin were scrapped off and extracted with methanol. Spectral studies were carried out on each isolated compounds (Table II).

Moreover, the NMR spectra for each of the trimethylsilylated compound [1] were typical of a flavonoid showing a similar pattern as those corresponding to luteolin-7-monoglucoside, apigenin-7-glucoside and quercetin respectively.

Hydrolysis of the isolated flavonoid glycosides (by refluxing with 20% H_2SO_4 for 6 hours) afforded glucose as the sugar moiety of each glycoside (identified by paper chromatography using butanol acetic acid-water 4 + 1 + 5 and aniline oxalate reagent as chromogenic spray [7]).

Results and Discussion

A. Nitrogenous bases

The hydro-methanolic extract of the defatted powdered plant was acidified with hydrochloric acid and extracted with benzene. The acidic layer was then alkalinised and extracted with chloroform. The organic layer gave negative test for alkaloids (common alkaloidal reagents). The alkaline phase was then adjusted to pH 4 with 10% hydrochloric acid, filtered (resinous matter). The extraction was proceeded according to the reineckate method [2]. After removal of the reineckate ion (by anion exchange resin), the methanol eluate was evaporated under reduced pressure and examined under reduced pressure and examined on TLC using silica gel G. (Methanol-water 1 + 1); two spots were revealed, having R_F values: 0.48 and 0.11 identical with authentic stachydrin and choline respectively. The identity of these two components as stachydrine and choline was confirmed, after their separation by preparative TLC and cochromatography of the isolated substances with the corresponding authentic material and their identical IR spectra, as well as their salts. The spot with R_F 0.48 gave an orange colour with Dragendorff's reagent, while that with R_F 0.11 gave a violet colour, characteristic reaction for choline [3].

B. Flavonoids

The ethyl acetate extract of the defatted powder revealed three flavonoid components on TLC using different solvent systems. The identity of these components as flavonoids was also confirmed (Shinoda test) [4].

The spots had the same R_F as authentic luteolin-7-monoglucoside, apigenin-7-glucoside and quercetin. Further identification was carried out on the isolated compounds by their colours detected in day light, UV light, and day light, UV light after treatment with ammonia (Table I); their maximum absorbance in methanol, and after addition of different reagents [5] (Table II).

The NMR spectra of the trimethylsilylated isolated flavonoids showed the same pattern as the corre-

Table I. R_F values of the isolated flavonoids and authentic references.

Flavonoids/authentics	R_F Silica gel G.		Colour			
	i	ii	DL	UV	NH_3 DL	NH_3 UV
I	0.08	0.06	y	p	y	y
II	0.32	0.16	y	p	y	y
III	0.37	0.30	y	y	y	y
Luteolin-7-monoglucoside	0.08	0.06	y	p	y	y
Apigenin-7-glucoside	0.32	0.16	y	p	y	y
Quercetin	0.38	0.30	y	y	y	y

i, Chloroform—methanol-formamide (80+19+1).
ii, Ethyl acetate—benzene—methanol—formamide (50+40+19+1).
y: yellow; p: purple.

Table II. Absorption maxima (nm) of the isolated flavonoids.

Addition of solvent (methanol)	Compound I		Compound II		Compound III	
	Band I	Band II	Band I	Band II	Band I	Band II
None	348	255	333	268	370	255
Sodium acetate	405	259	387	267	390	274
Boric acid + Sodium acetate	372	259	340	267	388	261
Aluminium chloride	432	274	386	276	458	272
Sodium methylate	394	263	386	269	321	247

sponding spectra of: luteolin-O-monoglucoside, apigenin-7-glucoside and quercetin. Hydrolysis of the first two glycosides showed that the sugar moeity is glucose.

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