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LIPIDS, PIGMENTS AND SAPONINS OF SANSEVIERIA CYLINDRICA, BOJER.

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ABSTRACT

Eleven fatty acids were identified by GLC analysis of the saponified fraction of the lipid content of S. cylindrica, Bojer leaves. A hydrocarbon and β- sitosterol were isolated from the unsaponifiable fraction and were identified by IR and GLC techniques.

The unsaponifiable fraction of the root contained B-carotin. The plant saponins were also isolated and estimated.

INTRODUCTION

Sansevieria cylindrica, Bojer is a herbaceous perennial plant benlonging to the Agavaceae family[1,2]. Agavaceae plants are used in folk medicine to promote healing, to act as a vermifuge for round and tapeworms and for the treatment of rheumatic fever, cough and T.B.[3-5].

Agavaceae family is characterized by its sapogenin content which was extensively studied. However, the other plant constituents have received little attention[6].

Preliminary phytochemical screening of the plant organs revealed the presence of unsaturated sterols and/or triterpenes, pigments and saponins. Sapogenins were investigated in previous puplication[7]. The present work is directed for studying of the lipids, pigments and saponins of that plant.

EXPERIMENTAL

1. Plants materials: The plants used were cultivated at the Faculty of Medicine, Tanta University and were identified by the late Dr. Vivi Tackholm, Prof. of plants taxonomy, Faculty of Science, Cairo University.
2. Chemicals and reagents were of analytical grade.

A. Investigation of Lipids:

I. Isolation: One Kg aliquots of each of the air-dried powdered leaves and subterranean organs of *S. cylindrica* Bojer were separately exhausted with petroleum ether (40-60°). The solvent was distilled off and the residue was refluxed with 0.5 N alcoholic KOH for 6 hours. The unsaponifiable matter was extracted with ether from the mixture after distillation of the alcohol. The ether was evaporated and the residue was dissolved in chloroform; the solution was used for the chromatographic investigation and separation of the unsaponifiable matter. The aqueous alkaline solution was acidified with H_2SO_4 and was exhausted with ether for separation of the fatty acids.

II. Chromatographic investigation of unsaponifiable matter:

a) TLC Studies:

Silica gel G plates were spotted with a chloroformic solution of the unsaponifiable matter alongside with *B*-sitosterol authentic sample. The chromatoplates were developed with benzene-ethyl acetate (9:1) ,I, and (4:1), II. The resolved spots were located by spraying of the plates with 10% H_2SO_4 solution in ethanol and heating at 120° for 10 minutes.

b) Column chromatographic separation:

A Silica gel column was used for the separation of 10 grams of unsaponifiable matter. Elution was started with benzene

and was completed with benzene-ethyl acetate mixtures adopting the gradient techniques. The pooled fractions were subjected to crystallization. The crystallized components were subjected to IR and GLC analyses.

III. Identification and Determination of Fatty Acids:

The methyl esters of the fatty acids, which were prepared by refluxing of the fatty acids with HCl in methanol for two hours[8], were analyzed by GLC, using a Varian model 3700 gas chromatograph equipped with a flame ionization detector. The column (2 M X ½ inch) was packed with 20 % DEGS adsorbed on chromosorb W (Mesh 100-120). Helium was used as a carrier gas at a flow rate of 30 ml/min. The temperatures were 180, 200 and 300° for column, injector and detector respectively; chart speed was 1 cm/min.

The fatty acids were identified by comparing their relative retention times with those of standard fatty acids[9].

B. Pigments:

TLC investigation of the root unsaponifiable matter revealed presence of an upper orange brown spot with R_f values of 0.95 and 0.87 using the solvent systems: I and II, respectively.

The content of the spot resolved was isolated from the unsaponifiable matter of the root by a column chromatographic technique using 5 gm of the unsaponifiable matter and hexane as the eluant. The eluate was evaporated and the residue was crystallized from benzene-methanol mixture (1:1). The crystallized deep red prisms were subjected to physical and spectral analyses.

C. Saponins:

I. Isolation: Three hundred grams of each of the defatted powdered leaves and subterranean organs were separately exhausted with ethanol. The ethanolic extracts were separately evaporated and the residues were exhausted first with chloroform then with ethyl acetate and finally with n-butanol; 5 x 100 ml portions in each

case. The combined n-butanol extract was evaporated till dryness and the residue was purified by dissolving in ethanol and by precipitation with ether several times until a white product of saponins was obtained.

ii. Hydrolysis: 10 mg of the purified saponins were refluxed with 20 ml of 10 % HCl for 3 hours on a steam bath. The mixture was exhausted with $CHCl_3$. The combined $CHCl_3$ extract was concentrated and was used for the chromatographic investigation of the aglycones. The aqueous solution was neutralized with Na_2Co_3, concentrated to a small volume and used for the sugar investigation.

iii. Chromatographic investigation: Silica gel G plates were used and were developed with butanol-ethanol-25 % aqueous ammonia (7:2:5) ,III, or isopropanol-water-formic acid((70:24:6) IV, for the curde saponins, and with benzene-ethylacetate (7:2) V or $CHCl_3$-MeOH (97:3),VI,for the 10 % H_2SO_4 solution in ethanol. Whatman No. I filter papers developed with n-butanol-glacial acetic-water were used for the investigation of the sugar moiteies. The resolved spots were located with aniline-hydrogen phthalate reagent.

iv. Quantitative estimation: The haemolytic index and the blood agar haemolytic zone methods[10,11] were used for the estimation of the saponin content of *S.cylindrica*, Bojer.

a) Haemolytic index determination: Ten grams of each of the powdered leaves and subterranean organs were exhausted by boiling with 100 ml of an isotonic buffer solution (pH 7.4)[10]. The mixture was filtered and the marc was washed two times each with 50 ml of the isotonic buffer solution. The filtrate and the washings were transferred to 250 ml measuring flask and the volume was adjusted to the mark with the buffer. Different aliquots (0.2 - 2 ml) of the solution were used for the determination of the hoemolytic index according to literature procedure[10].

b) The blood agar haemolytic zone method depends on the formation of a circular zone of haemolysis produced by a saponin solution placed in special cups in blood agar plates. The dried powdered leaves and subterranean organs were separately extracted by boiling with distilled water for 30 minutes. The mixture, in each case, was filtered and the filtrate was completed to 100 ml with distilled water and was sterilized by bacterial filtration. A volume (0.1 ml) of the sterile solution was assayed according to literature[11] procedure.

RESULTS AND DISCUSSION

A. Lipids:

The TLC investigation of the unsaponifiable matter revealed the presence of 9 spots; two of which were isolated by column chromatography and were identified by thermal and chemical analyses as β-sitosterol and as saturated hydrocarbons (Table 1).

The GLC analysis of the fatty acid methyl esters revealed the presence of 15 fatty acids (Table 2). The Table indicates that the saponifiable fraction of the lipids of *S. cylindrica* leaves is formed mainly of linolenic acid (40.1%) all of the fatty acids, except three, were identified and quantitated.

B. Pigments:

The isolated substance existed as deep red prisms. It melted at 183° and exhibited λ maxs at 466 and 497 nm in $CHCl_3$ and at 440 and 495 nm in hexane. Its solution in ethanol had a strong fluorescence with maximum excitation and emission at 345 and 410 nm, respectively .

This result was in agreement with that reported by Cherry et al[12]. The IR of that pigment showed peaks at 1715 and 1660 cm^{-1}. These peaks were superimposing with those obtained from the IR spectrum of suthentic β-carotin.

C. Saponins:

The TLC investigation of the saponins, separated from the leaves and the subterranean organs, revealed the presence of 3 spots having R_f values of 0.59, 0.48 and 0.43 on using solvent III; with solvent IV, two spots with R_f values of 0.24 and 0.19 were observed.

The TLC investigation of the aglycones of the hydrolysed saponins showed 9 spots, three of which showed R_f values of 0.6, 0.56 and 0.31 on using solvent system VI, and 0.7, 0.62 and 0.13 on using system V; the resolved spots were chromatographically identical with β-sitosterol, diosgenin and ruscogenin respectively.

Paper chromatography of the solutions containing the sugar moieties revealed the presence of arabinose, galactose and other two unidentified sugars.

The haemolytic index of the saponins separated from the leaves and the subterranean organs were 1/500 and 1/250, respectively. The saponin contents of the leaves and the subterranean organs, determined by blood-agar heamolytic zone method, were 3.54 and 1.31 percent, respectively.

Table (1) : TLC, Thermal and Chemical Analyses of the Unsaponifiable Matter of S. cylindrica, Bojer Leaves.

Compound	R_f using solvent		Melting Point, °C	IR	GLC	Identification
	I	II				
A	0.83	0.92	78	Peaks at 2950,2840 and 1480 cm^{-1}, no peaks indicating special functional groups (OH or C=O) were observed.	19 hydrocarbon components [13]	Saturated Hydrocarbons.
B	0.31	0.51	135-137	superimosed with that of authentic β-sitosterol.	The acetate derivatives showed two peaks after retention times of 22 and 27 minutes.	β-sitosterol (mainly)

Table 2. Fatty Acids Present in S. cylindrica, Bojer Leaves.

Peak No	Relative retention time	Identification	Percentage
1	0.09	lauric	7.4
2	0.18	unknown	11.9
3	0.23	myristic	1.8
4	0.29	iso-pentadecanoic	6.3
5	0.39	iso-palmitic	2.9
6	0.49	palmitic	2.0
7	0.55	palmitoleic	1.2
8	0.84	iso-stearic	8.5
9	1.00	stearic	1.6
10	1.10	oleic	0.1
11	1.52	linolenic	40.1
12	1.80	unknown	0.90
13	2.30	gadoleic	5.6
14	3.06	unknown	7.2
15	3.87	arachidonic	traces

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الدهنيات والصيغات والصابونين
لنبات السانسفيرا سلندريكا بوجر

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تم التعرف فى هذا البحث على احدى عشر حمض دهنى فى الجزء المتصبن من دهنيات اوراق هذا النبات بواسطة كروماتوجرافيا الغاز. كما تم فصل مادة البيتاسيتوستيرول وكذلك مادة كربوهيدراتية مشبعة من الجزء الغير متصبن من نفس الدهون بواسطة كروماتوجرافيا العمود وحللت المواد المنفصلة بواسطة كروماتوجرافيا الغاز والاشعة تحت الحمراء.

كما فصلت مادة البيتاكاروتين من الجزء الغير متصبن من الدهن الموجود فى جذور النبات.

وقد تم فصل مادة الصابونين من الاوراق والجذور والريزومات وحلل هذا الصابونين الى مكوناته الاساسية (صابوجنين ، سكريات) وتم تعيين نسبته بطرق مختلفة ووجد ان الاوراق تحتوى على نسبة منه أعلى من تلك الموجود فى الجذور والريزومات.

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