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A COMPARATIVE PHYTOCHEMICAL STUDY OF THE LIPID CONTENT OF *IBERIS GIBRALTARICA* L. AND *IBERIS UMBELLATA* L. CULTIVATED IN EGYPT

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ABSTRACT

Gas liquid chromatographic (GLC) analysis of the fatty acids of *Iberis gibraltarica* , revealed the presence of 15 compounds, 11 of which were identified. Plamitic, stearic and oleic acids were the major compounds (88.83%). While in *Iberis umbellataa*, the same study revealed the presence of 16 compounds, 12 of which were identified. Lauric (only identified in *I. umbellata*), palmitic, stearic and oleic constituted about (74.23%) of the total fatty acid content.From the unsaponifiable matter of *I. gibraltarica* and after acetylation the 4-demethyl sterol (26, 27-Dinorergosta-7, 22-dien-3B-yl acetate) 1 was isolated, identified on basis of spectroscopical evidence.

INTRODUCTION

The family Cruciferae is a large family with metabolites of pungent or acrid odour and tase, but not poisonous plants (1). Many members of the family demonstrated wide application in folk medicine; *Iberis amara* (bitter candytuft) recommended strongly in sciatica and the seeds are employed in haemopathy as a heart tonic(2).

The distribution of cucurbitacins in seeds and growing plants of *I. gibraltarica* L., *I. umbellata* L., and other crucifers was reported(3).

In previous report(4), a comparative macro-and micromorphological study of the leaf and stem of *I. gibraltarica* and *I. umbellata* was given.

Screening the available literature, nothing was found dealing with the study of lipid content of both plants. Thus, the present work was undertaken.

EXPERIMENTAL

1. Materials:

Samples of both plants were collected in April 1994 from the plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University. The entire plants were dried and reduced to powder No. 36.

2. Apparatus:

Melting point was determined on a Reichert melting point microscope. IR spectrum was obtained in KBr discs using Shimadzu infrared spectrophotometer IR-435. ^1H & ^{13}C NMR spectra were recorded at 90 MHz on a Jeol-FX90Q NMR spectrometer in $CDCl_3$ using TMS as internal standard. MS was recorded on HP Model: MS 5988, Inlet type: Dip, Electron energy 70 eV. Analytical TLC was carried out on silica gel 60 GF, while PLC was on silica gel 60 PF_{254} (0.5 mm, prepared in 5% $AgNO_3$).

GLC analysis of fatty acid methyl esters was conducted on a Perkin-Elmer Sigma-3B gas chromatograph equipped with Perkin-Elmer-2 calculating integrator; a dual flame ionization detector and a coiled glass column (1.5 m x 4 mm) packed with 3% SE-30, adsorbed on 100-120 mesh diatomite. Oven temperature was programmed at 10°C/min from 100-250°C, then isothermally at 250°C for 30 min. Nitrogen flow rate was 30 ml/min. Injector and detector temperatures were 270 and 280°C, respectively. Chart speed was 30 cm/hr.

The identification of the individual components was carried out by comparing their retention times with those of the available authentic samples. The quantitative estimation of each compound was carried out based on measurement of peak area. The results are listed in the given table (1).

Investigation of the lipid content:

The powder of each plant (1 Kg) was separately extracted to exhaustion with petroleum ether (b.r. 60-80°C) in a Soxhlet apparatus, to yield 21.6 g (for *I. gibraltarica*) and 19.3 g. (for *I. umbellata*) of dark greenish brown residues.

Preparation of the unsaponifiable matter:

The residue obtained from the petroleum ether extract of each plant was separately saponified with methanolic potassium hydroxide (40%) for 24 hr. at room temperature(5). The unsaponifiable fractions were extracted with ether. The ethereal extracts were separately evaporated to dryness to yield (12 g. for *I. gibraltarica*) and (10.5 g. for *I. umbellata*).

TLC. investigation of the (USM) of both plants using solvent system toluene: chloroform (7:3) and spraying reagent 50% H_2SO_4 in ethanol; showed similarity between the components of the USM of both plants. Therefore, further work was carried out on USM of *I. gibraltarica* only. The (USM) about 12 g. was adsorbed onto a column of silica gel 60 (400 g.). The material was chromatographed using the following solvents: toluene; toluene-chloroform (1:1); chloroform-toluene (7:3); chloroform; chloroform: ethanol (1:1) and ethanol; 47 fractions of 250 ml were collected. Similar fractions were grouped together according to TLC examination. Fractions 13-24 (2.80 g. collected by elution with toluene-chloroform 1:1) was acetylated with pyridine-acetic anhydride and the resulting steryl acetates (1.3 g.) was subjected to preparative layer chromatography on silver nitrate plates (using solvent system Hexane-Ethyl acetate 99:1, run 3X, spray H_2SO_4 50% in ethanol, which allowed isolation of one major crystalline compound (350 mg),

m.p 133-136°C [Lit.[6] 135-138°C]; IR ν_{max} cm^{-1}: 1735, 1250 (OAc); ^1H NMR: δ(90 MHz, $CDCl_3$): 0.51 (3H, s, C-18), 0.80 (3H, s, C-19), 0.92 and 0.99 (d, C-25 and C-26), 1.04 (d, C-21), 2.16 (s, 3B-OAc), 4.7 (m, 3 α H), 5.16 (m, C-7), 5.25 (m, C-22 and C-23); ^{13}C NMR: δ(90 MHz, $CDCl_3$), C-1 (37.40), C-2 (25.60), C-3 (72.20), C-4 (33.60), C-5 (39.20), C-6 (29.20), C-7 (118.40), C-8 (137.40), C-9 (48.40), C-10 (35.20), C-11 (22.40), C-12 (38.40), C-13 (42.20), C-14 (58.80), C-15 (24.40), C-16 (27.60), C-17 (59.60), C-18 (11.80), C-19 (12.80), C-20 (40.40), C-21 (20.40), C-22 (135.60), C-23 (126.40), C-24 (52.40), C-25 (30.80), C-26 (29.60), $OCOCH_3$ (169.20), $OCOCH_3$ (20.80). MS : m/z (rel. int.): 412 (2, M+), 397 (100), 394 (23), 382 (64), 367 (16), 352 (2), 340 (4), 255 (34), 213 (25), 149 (69), 81 (55).

Preparation of fatty acid methyl esters:

The remaininng aqueous solution of each plant was acidified separately with hydrochloric acid and the liberated fatty acids were extracted with ether and their methyl esters were prepared[7] and subjected for G.L.C. analysis. Results are recorded in Table (1).

RESULTS AND DISCUSSION

Column and PLC of the unsaponifiable matter of *I. gibraltarica* has led to the separation of a substance with m.p 133-136°C (350 mg.). It's structure is assigned on the basis of spectroscopic analysis.

Mass spectrum showed [M+] peak at m/z 412, and significant peaks at m/z 397 and 382 due to loss of two methyl groups. The peak at m/z 394 arises due to dehydration of the compound. Peak at m/z 352 for loss of acetate. Peaks at m/z 255 for loss of the side chian and of acetate and at m/z 213 (255-42) gave indication of the presence of Δ^7 sterol. Explanation for the presence of m/z 149 ion is that C-8 is contained in the charged fragment but provides the hydrogen atom which is transferred during the cleavage[8]. This interpretation necessitates breaking of two bonds attached to C-8 and one possibility is indicated in the following sequence:

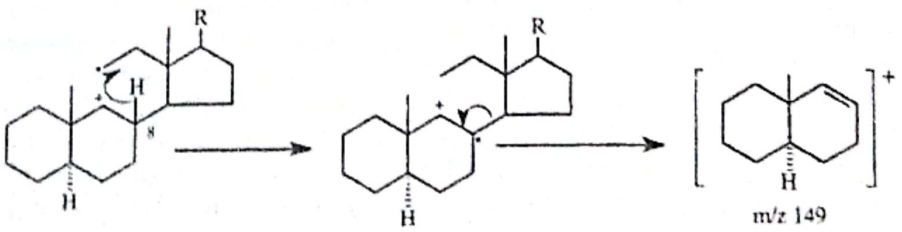
The ^1H NMR spectrum shows the expected chemical shifts[6] for the signals due to the protons of the C-18 and C-19 methyl groups at δ0.51 and 0.80 respectively (Lit at δ0.54 and 0.82 for C-18 and C-19 respectively). The doublets at δ0.92, 0.99 and 1.04 (Lit at δ0.93, 0.97 and 1.03 for C-25, C-26 and C-21 respectively). The rest of signals were the multiplets at δ4.7, 5.16 for 3α-H and C-7 respectively (Lit at 4.65 and 5.18). The acetoxy methyl signal appears at 2.16 (Lit.[6] at δ2.01).

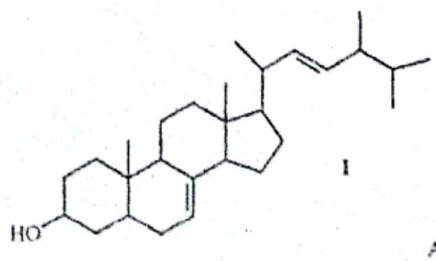
The ^{13}C NMR specteum shows the presence of 28 carbon atoms in the molecule. The lowest field methine resonances at δ135.60, 126.40, 118.40 and 72.20 were assigned to C-22, C-23, C-7 and C-3 respectively by comparing with Δ^7 related compounds[9]. The lowest field quaternary resonance δ137.40 was assigned to C-8. Among the methylene resonances the lowest field one at δ38.40 was attributed to C-12.

Thus, the spectroscopic data of the isolated compound confirmed its structure as 26, 27-Dinorergosta-7, 22-dien-3B-yl acetate B. [The nomenclature 26, 27-Dinorergosta means that this compound lacks the two methyl groups at C-26 & C-27 in comparison with the parent compound 2 (5-Dihydroergosterol; 5α -ergosta-7, 22-dien-3B-ol)[10]].

G.L.C analysis of the fatty acid methyl esters of lipid content of *I. gibraltarica* and *I. umbellata* shows identity of the fatty acids except lauric acid which is present only in *I. umbellata* as shown in Table (1). The main fatty acids of both plants are saturated with lesser amount of unsaturated ones. Those of *I. gibraltarica* are palmitic (41.43%), stearic (14.96%), lauric (10.36%) and oleic (7.48%).

This is the first report about these constituents from these plants.





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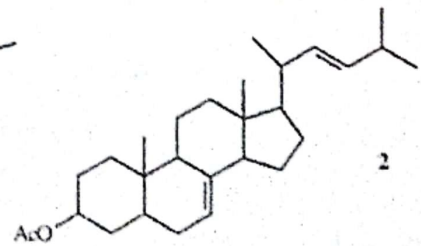

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Table (1): GLC analysis of the fatty acid methyl esters of lipid content of *I. gibraltarica* and *I. umbellata*.

Peak No.	Authentics methyl esters of	RR* Ib.G	RR* Ib.U	Relative Percentage Ib.G	Relative Percentage Ib.U
1	Caproic acid	0.16		0.21	0.69
2	--	0.29		0.28	0.23
3	Nonaoic acid	0.41		0.07	0.29
4	Capric acid	0.51		0.08	0.26
5	Undecanoic aicd	0.67		2.29	4.03
6	Lauric acid	--	0.71	--	10.36
7	Tridecanoic acid	0.85		0.90	4.72
8	Myristic acid	0.92		0.69	1.04
9	Palmitic aicd	1.00		33.26	41.43
10	Heptadecanoic acid	1.06		0.76	0.69
11	Stearic acid	1.12		49.06	1.96
12	Oleic acid	1.15		6.51	7.48
13	--	1.19		1.04	6.79
14	--	1.26		1.18	1.15
15	Linoleic acid	1.37		1.59	2.07
16	--	1.73		2.07	3.80

* Retention time relative to methyl palmitate (R_t=14.60);
---=Unidentified; Ib. G=*Iberis gibraltarica*; Ib.U = *Iberis umbellata*

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دراسة كيميائية مقارنة للمحتوى الدهنى لكل من نباتى الايبيرس جيبرلاتريكا (لينية) والايبيرس امبيلاتا (لينية) والمنزرعان فى مصر

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سبق إجراء دراسة مقارنة للصفات العيانية والمجهرية لاوراق وسيقان كل من نباتى الايبيرس جيبرلاتريكا (لينية) والايبيرس امبيلاتا (لينية). ويشتمل هذا البحث على دراسة الاحماض الدهنية المحضرة من كلا النباتين لاول مرة. وتم التعرف على ١١ مركبا من نبات الايبيرس جيبرلاتريكا، تمثل أحماض بلمتك، ستياريك، والاوليك ٨٣ . ٨٨٪ من المحتوى الدهنى. فى حين تم التعرف على ١٢ مركبا من نبات الايبيرس امبيلاتا منها احماض اللاوريك، بلمتك، ستياريك، والاوليك والتى تكون ٢٣ . ٧٤٪ من المحتوى الدهنى. كذلك تم تحليل المواد الغير متصبنة فى نبات الايبيرس جيبرلاتريكا، وتم فصل استيرول ٢٦، ٢٧ - داينورارجوستا - ٧، ٢٢ - دايين - ٣ بيتايل اسيتات لاول مرة من هذا النبات. وتم التعرف على التركيب الكيميائى لهذه المادة عن طريق درجة الانصهار، تحليل طيف الكتلة، التردد النووى المغناطيسى وكذلك الاشعة تحت الحمراء.