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TRITERPENES AND FLAVONOIDS FROM *FAGONIA MOLLIS* DEL VAR. *GRANDIFLORA*

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تم فى هذا البحث فصل أربعة مركبات ثلاثية التربين وهى ليوبيول ، ليوبيول أسيتات ، ليوبيول بالميتات والمركب الرابع لم يتم التعرف عليه وكذلك تم فصل أربعة مركبات فينولية وهى كامبفيرول ، هيرباسيتين-٨-ميثيل ايثر ، أيزورامنيتين وكوارسيتين وجميع هذه المركبات تفصل لأول مرة من النبات موضوع البحث وقد تم التعرف عليها باستخدام الطرق الطيفية المختلفة.

The aerial parts of the Fagonia mollis var. grandiflora afforded lupeol, lupeol acetate, lupeol palmitate and unidentified triterpene together with four flavonoids kampferol, herbacetin-8-methyl ether, isorhamnetin and quercetin. Triterpenes were identified by ^{1}H-NMR, ^{13}C-NMR and mass spectral analysis and flavoloids by U.V., ^{1}H-NMR and mass spectral analysis.

INTRODUCTION

The genus *Fagonia* (Family Zygophyllaceae) is represented in Egypt by 18 species,[1] among which is *Fagonia mollis* var. grandiflora. In traditional medicine, extracts of *Fagonia* species were reported to exhibit medicinal properties and used for treatment of cholera, prolonged fever and snake bites.[2,3] Also, it was reported that a mixture of saponins obtained from the 60% aqueous ethanolic extract of *Fagonia mollis* complex exhibited anti-inflammatory and antipyretic activities, but a weak analgesic effect.[4]

A literature survey revealed that this genus is rich in flavonoids and saponins.[5-10] However nothing have been reported on the isolation of the active constituents of the plant under investigation. It is the aim of this study to investigate flavonoids and triterpenes in this plant.

EXPERIMENTAL

Plant material

The plant was collected from Sinai desert near El-Arish. It was identified and authenticated by Prof. Dr. A. Fayed, Professor of Plant Taxonomy, Dept. of Botany, Faculty of Science, Assiut University. The aerial parts were air-dried, powdered and kept in well closed dark container till used.

General procedures

Melting points were uncorrected and determined with Koffler hot stage microscope. UV spectra were recorded using Unicam 1750 spectrophotometer with Pye-Unicam Ar 55 linear recorder. Varian $JM_3NGX500$ spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) in $CDCl_3$, DMSO-d_6 and JEOL JMS-DX 300 L Mass spectrometer for MS measurements. Silica gel (E. Merck) was used for column chromatography for separation of triterpenes. Silica gel G60 was used for monitoring of triterpenes using hexane-ethyl acetate (85:15 v/v) as solvent system, while Whatman No. 3. paper chromatography was used for separation of flavonoids using system: n-butanol-acetic acid-water [BAW (4:1:2 v/v)].

Isolation and characterization of compounds

The powdered air-dried aerial parts (2 Kg) were extracted with methanol till exhaustion. The extract was concentrated under reduced pressure to syrupy consistency, mixed with

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water and successively extracted with hexane and chloroform. Both hexane and chloroform fractions were subjected to chromatographic examination for their contents.

The hexane fraction (10 g) was subjected to column chromatography using hexane-EtOAc gradient, where compound **1** was eluted with hexane-ethyl acetate (95:5), further fractions using the same polarity gave compound **2**, while further elution using hexane-EtOAc (94:6) gave compound **3**, elution with hexane-EtOAc (92:8) gave compound **4**.

The chloroform fraction (8 g) was subjected to column chromatography on silica gel using chloroform-methanol gradient. The similar fractions were collected and subjected to paper chromatography using n-butanol-acetic acid-water (4:1:2) and 15% acetic acid as solvent systems. Preparative paper chromatography was used to separate the components, where four compounds were isolated F1-F4.

Compound 1: needles from acetone, m.p. 210-212°C. EIMS, m/z 426, other fragments: 407, 411, 218, 207 and 204. ^{1}H-NMR: δ 3.19 (1H, dd, J= 5.0, 11.0 Hz, H-3), 2.38 (1H, ddd, J= 5.95, 11.0, 11.0 Hz, H-19), 0.93 (3H, s, H-23), 0.75 (3H, s, H-24), 0.82 (3H, s, H-25), 1.03 (3H, s, H-26), 0.96 (3H, s, H-27), 0.78 (3H, s, H-28), 4.56 (1H, s, H-30), 4.68 (1H, s, H-30), 1.68 (3H, s, H-29). ^{13}C-NMR (Table 1).

Compound 2: needles from methanol, m.p. 215-218°C. EIMS, m/z 468, other fragments: 453, 249, 218, 209, 203 and 189. ^{1}H-NMR: δ 4.50 (1H, dd, J= 8.4, 4.80 Hz, H-3), 2.38 (1H, ddd, J= 5.98, 11.07, 11.07 Hz, H-19), 0.81 (3H, s, H-23), 0.82 (3H, s, H-24), 0.83 (3H, s, H-25), 1.04 (3H, s, H-26), 0.95 (3H, s, H-27), 0.78 (3H, s, H-28), 1.66 (3H, s, H-29), 4.58 (1H, br.s, H-30), 4.69 (1H, br.s, H-30) and 2.1 (3H, s, COC$\underline{H}_3$). ^{13}C-NMR (Table 1).

Compound 3: Amorphous powder, m.p. 230-232°C (from methanol). EIMS m/z: 664, fragments, 649, 621, 425, 408, 218 and 189. ^{1}H-NMR: δ 0.88 (3H, t, J= 6.9 Hz, C$\underline{H}_3$-terminal of fatty acid), 1.28 (m, C$\underline{H}_2$-residue of fatty acid), other protons as in lupeol. ^{13}C-NMR (Table 1).

Compound 4: Was obtained as amorphous powder, m.p. 227-229°C (from methanol). EIMS m/z: 482, other fragments, 453 (M-CHO), 298, 263, 249, 218, 189, 175 and 161. ^{1}H-NMR: δ 9.00 (1H, s, C$\underline{H}$O), other signals similar to lupeol acetate. ^{13}C-NMR (Table 1).

Compound F1: Yellow needle (MeOH), m.p. 282-284°C. EIMS m/z: (rel. int.%) 286 (60), 258 (48), 153 (3) and 152 (5). ^{1}H-NMR (Table 2) and U.V. (Table 3).

Compound F2: Yellow amorphous powder (MeOH), m.p. 269-271°C. EIMS m/z: (rel. int.%) 316 (100), 315 (55), 301 (11), 167 (8), 121 (13) and 93 (3). U.V. (Table 3).

Compound F3: Yellow amorphous powder, m.p. 308-310°C. EIMS m/z: (rel. int.%) 316 (25), 301 (5), 230 (6), 149 (15) and 121 (8). ^{1}H-NMR (Table 2) and U.V. (Table 3).

Compound F4: Yellow needle (MeOH), m.p. 314-316°C. EIMS m/z: (rel. int.%) 302 (100), 286 (70), 274 (20), 152 (5), 137 (30) and 122 (15). ^{1}H-NMR (Table 2) and U.V. (Table 3).

RESULTS AND DISCUSSION

From the aerial parts of *Fagonia mollis* V. grandiflora four triterpenes **1-4** were isolated from the hexane fraction, while four flavonoid aglycones were isolated from the chloroform fraction. The identification of these compounds was based on the study of physical, chemical, chromatographic as well as the spectroscopic data (UV, ^{1}H-NMR, ^{13}C-NMR and Mass spectra).

Compound 1: was chromatographically non identical with authentic samples of α and/or ß-amyrin. In the Mass spectrum, the molecular ion was detected at m/z 426 and other fragments at m/z 218 and m/z 207 which were derived from a Retro-Diels-Alder fragmentation for a

Table 1: The ^{13}C-NMR chemical shifts of compounds **1-4** ($CDCl_3$).

	1	2	3	4		1	2	3	4
C-1	38.7	38.7	38.7	38.9	C-20	150.9	150.9	151.2	151.2
C-2	23.8	27.4	25.0	27.4	C-21	29.8	29.8	30.0	30.0
C-3	81.2	78.9	81.2	78.9	C-22	40.0	40.0	40.2	40.2
C-4	38.8	38.8	39.0	55.9	C-23	28.0	28.0	28.2	22.1
C-5	55.3	55.3	55.5	48.0	C-24	15.4	15.4	15.6	200.1
C-6	18.2	18.3	18.5	21.0	C-25	16.1	16.1	16.3	16.3
C-7	34.2	34.2	34.7	34.7	C-26	15.9	15.9	16.2	16.2
C-8	40.8	40.8	41.0	41.0	C-27	14.5	14.5	14.7	14.7
C-9	50.4	50.4	50.6	50.6	C-28	18.0	18.0	18.2	18.2
C-10	37.2	37.1	37.2	37.2	C-29	19.3	19.3	19.5	19.5
C-11	20.9	20.9	21.1	21.1	C-30	109.3	109.3	109.5	109.5
C-12	25.1	25.1	25.3	25.3	Ac	21.3			21.3
C-13	38.0	38.0	38.3	38.3		173.6			173.6
C-14	42.8	42.8	43.2	43.2	F.A.				
C-15	27.4	27.4	27.6	27.6	$\underline{CH_3}$-T			14.3	
C-16	35.5	35.5	35.8	35.6	$\underline{CH_2}$-CH_3			22.4	
C-17	43.0	43.0	43.0	42.8	$\underline{CH_2}$-C=O			34.1	
C-18	48.2	48.2	48.5	48.3	$\underline{CH_2}CH_2C$=O			24.6	
C-19	47.9	47.9	48.2	48.2	CH_2 of F.A.			32.1	
								29.5-29.9	
					CH_2-$\underline{C}$=O			174.6	

F.A. = Fatty acid; Ac = Acetate; T. = Terminal

Table 2: ^1H-NMR data of the flavonol, F1 ($CDCL_3$), F3 and F4 (DMSO-d_6)

Compound	H-2'	H-6'	H-3'	H-5'	H-6	H-8	Ome
F1	8.2, d, J=2.5	8.2, d, J=2.5	6.95, d, J=2.5	6.95, d, J=2.5	6.54, d, J=2.5	6.30, d, J=2.5	----
F3	7.6, d, J=2.5	7.5, d, J=2.5	------	6.95, d, J=9.0	6.44, d, J=2.5	6.22, d, J=2.2	3.62, s
F4	7.66, d, J=2.0	7.54, d, J=2.8	------	6.97, d, J=2.5	6.41, d, J=2.5	7.19, d, J=2.0	----

Table 3: UV spectral data of the isolated compounds F1-F4.

Comp. / Reagent		MeOH	+$AlCl_3$	+$AlCl_3$ +HCl	+ NaOAc	+NaOAc +H_3BO_3	+NaOMe	R_f values in BAW (4:1:2)
F1	I	365 320	420 350 302	420 347 302	385 302	371 318	415 316	0.79
	II	265	267	268	271	264	277	
F2	I	377 325	430 355 310	429 356 308	400 340 318	380 320 308	425 dec.	0.78
	II	255	275	273	280	274	288	
F3	I	370 305	430 358 300	427 355 270	390 (dec.) 318	375 325 305	435 (dec.) 320	0.68
	II	251	262	260	272	253	270	
F4	I	370	438 363 303	491 360 304	395 327	392 327	410 (dec.)	0.58
	II	256 269	272	266	273	260	252	

triterpenes unsubstituted in C, D and E rings (Fig. 1) and having a hydroxyl group at the lower part of skeleton.[11] ^{1}H-NMR showed two olefenic protons at δ 4.56 and δ 4.68 (each 1H, brs) and a downfield olefenic methyl groups at δ 1.68 for either taraxasten or lupene skeleton. The ^{13}C-NMR showed its identify to lupeol rather than taraxasterol (Table 1). This was confirmed by comparing the ^{13}C-NMR data with that of lupeol.[12]

Compound 2: The mass spectra of this compound exhibited a molecular ion [M^+] at m/z 468 and gave fragmentation pattern similar to that of compound **1**. However, the fragment ion peak at m/z 207 in **1** shifted to m/z 249 in **2** indicating additional acetate group (CH_3CO) to the compound **1** which was confirmed by the ^{13}C-NMR peaks at δ 173.6 and δ 21.3 and downfield shift of H_3 in **1** from δ 3.19 (1H, dd, J= 9.4, 4.2 Hz) to δ 4.5 (1H, dd, J= 8.4, 4.8 Hz) in **2**, all of these data as well as the comparison of ^{13}C-NMR with that of lupeol acetate,[12] (Table 1). Suggested that compound **2** was lupeol acetate.

Compound 3: showed in ^{13}C-NMR, besides a lupene skeleton, a cluster of methylene peaks at δ 29.2-30, a methyl group at δ 14.3, a methylene group at 32.1 and a carbonyl peak at δ 174.6 ppm indicating possible presence of a fatty acid ester of lupeol. The EIMS showed a very weak peak at m/z 664 followed by a peak at m/z 649 (M-15) followed by successive loss of 14 mass unit. From the above data the compound was identified as lupeol palmitate which has not been previously isolated from the genus *Fagonia*.

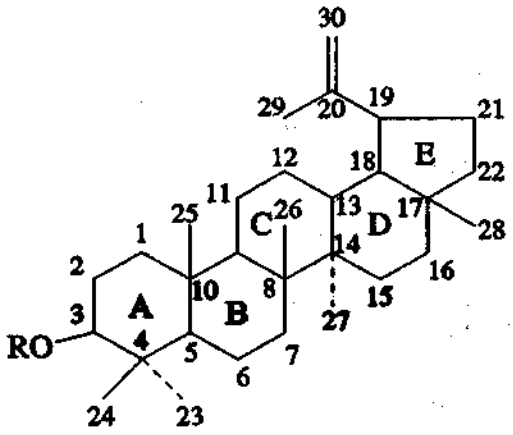

Fig. 1

1: R= Ac; 2: R= H;
3: R= Pal. O=C-$(CH_2)_{14}CH_3$; 4: unidentified

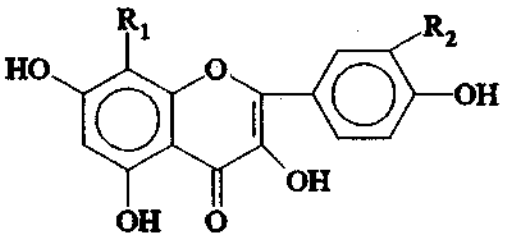

Fig. 2

	R_1	R_2
Compound F1	H	H
Compound F2	OCH_3	H
Compound F3	H	OCH_3
Compound F4	H	OH

Compound 4: gave similar ^{1}H-NMR and ^{13}C-NMR to lupeol acetate. However, its ^{1}H and ^{13}C-NMR showed a peak at δ 9.0 and at δ 200, respectively corresponding to the presence of an aldehydic function in compound 4. This was confirmed by MS which showed M^+ at m/z 482 and RDA fragmentation at m/z 263 and m/z 218 indicating the presence of aldehydic and acetate groups in A/B rings of this lupene derivative. This group may be located probably on C_{24} due to its chemical shift (C_{24} aldehydic group) appears more downfield. However, due to lack of more information, the structure of this compound is still uncertain.

Compounds F1 and F4 (Fig. 2): were identified as kampferol and querctin respectively, by studing their physical, chemical and UV spectral data with different ionizing and complexing agents.[13-15] (Table 3) as well as ^{1}H-NMR spectra (Table 2).

Compound F2 (Fig. 2): was obtained as a yellow amorphous powder, m.p. 269-271°C. The mass spectrum of F2 exhibited a molecular ion at m/z 316 in accordance with a flavanol containing four hydroxyls and one methoxyl group. The bathochromic shift (+48 nm) of band I in NaOMe relative to band I in MeOH with increase in intensity indicated the presence of a hydroxyl group at C-4. The presence of OH at C-7 was indicated by bathochromic shift (+25 nm) of band II in NaOAc relative to band II in methanol.[16] The absence of orthodihydroxyl group in ring B indicated by the bathochromic shift in band I (+3) in NaOAc/H_3BO_3 relative to band I in methanol. The presence of a hydroxyl group at C-5 was evident since the compound appears as a purple spot on paper chromatogram when viewed in UV light. According to the above data compound F2 was deduced as herbacetin-8-methyl ether.

Compound F3 (Fig. 2): was obtained as a yellow amorphous powder, m.p. 308-310. The ^{1}H-NMR spectrum (DMSO-d_6) confirmed the presence of one methoxyl group (δ 3.62) in addition, NMR signals (Table 2) were observed for five aromatic protons in accordance with isorhamnetin type flavanol (δ 7.6, 7.5 and 6.95 for B-ring and 6.4 and 6.2 for the A-ring protons). The presence of a hydroxyl group at C-5 was evident since the compound appeared as a purple spot on the paper chromatogram when viewed in UV light. The presence of a second hydroxyl group at C-7 was indicated by a bathochromic shift (+21 nm) of band II in NaOAc, relative to band II in methanol.[16] The bathochromic shift in band I (+5 nm) and (+2 nm) band II with NaOAc/H_3BO_3 indicating the absence of a free orthodihydroxyl group in ring B. So compound F3 was identified as isorhamnetin.

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