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SESQUITERPENE LACTONES AND FLAVONOIDS FROM *INULA BRITANNICA* (L.)

Omar M. A. El-Towesy

Deptartment of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

نبات الانيولا بريتانيكا ل. من النباتات التابعة للفصيلة المركبة والمشهورة باحتوائها على لاكتونات السسكتربين وأسفرت هذه الدراسة عن فصل خمسة مواد لاكتونية وهى:

١- ٤-ألفا-٥-بيتا ايبوكسى ايوباتوليد.
٢- ٤-ألفا-٥-بيتا ابيوكسى ديس استيل اوفاتفولين.
٣- ٥-الفا-هيدروكسى ديهدرو ليوكودين.
٤- ١٤-هيدروكسى-٢-اوكسوجويا-١(١٠)-٣-دين-٥-الفا-١١-بيتا-هـ-٦،١٢ الفا اوليد
٥- ٢-أوكسو-٨ الفا و ١٠بيتا-داى هيدروكسى جويا-٣-اين-١ ألفا و ٥ ألفا-٦ بيتا و ١١ بيتا هـ-١٢ ، ٦ اوليد.

هذا بالإضافة إلى ثلاثة مواد فلافونيدية هى: أبيجنين وابيجنين-٧-أ-جلوكوزيد وكورستين ٣"،٤" داى ميثوكسى-٧-أ-جلوكوزيد.

From Inula britannica (L.) five known sesquiterpene lactones were isolated and identified as 4α,5β epoxyeupatolide (1); 4α,5β epoxydesacetylovatifolin (2); 5α-hydroxydehydroleucodin (3); 14-hydroxy-2-oxoguaia-1(10),3-dien-5α,11BH-12,6α-olide (4) and 2-oxo-8α,10β-dihydroxyguai-3-en-1-α,5α,6β,11βH-12,6-olide (5) in addition to three known flavonoids viz. apigenin (6), apigenin-7-O-β-glucopyranoside (7) and quercetin 3',4'-dimethoxy 7-O-β-glucopyranoside (8). The structures of these compounds were depicted through spectral studies. All the isolated compounds were reported for the first time from the mentioned plant.

INTRODUCTION

Inula britannica (L.) is a perennial plant belonging to family *Asteraceae*.[1] This plant is prescribed as a remedy for cough, vomiting and treatment of sore throat.[2] Previous investigation of *inula* species gave in addition to some common compounds,[3] several sesquiterpene lactones.[4] As a part of our research program dealing with sesquiterpene lactones of compositae.[5-7] We examined *Inula britannica* (L.) from which five sesquiterpene lactones and three flavonoids were isolated and identified.

EXPERIMENTAL

All mps are uncorrected. EIMS were recorded at 70 ev. NMR spectra were recorded at 200 and 50.3 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are in δ value with TMS as internal standard. TLC carried out on silica gel using solvent systems:
System I: Benzene-Acetone (8:2)
System II: Chloroform:Methanol (9.5:0.5)
System III: Chloroform:methanol (7:3)

Spots were visualized by their fluorescence at 254 nm in U.V. or by spraying with 5% H_2SO_4 (for sesquiterpenes), $AlCl_3$ (for flavonoids) and aniline hydrogen phthalate (for sugars).

Plant material

The plant material collected during the flowering stage in April 1995 from the Bontanical Garden of Faculty of Agriculture, Assiut University, identity was confirmed by Prof. Dr. A. Fayed, Professor of Taxonomy, Faculty of Science, Assiut University. Voucher specimens are deposited in the Herbarium of the Department of Pharmacognosy.

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Extraction and isolation

The air-dried powdered aerial parts of the plant (2 kg) was defatted with petrol (40-60°C) followed by extraction with methanol by percolation. The concentrated methanolic extract (60 g) was fractionated with chloroform to give fraction A (20 g) and the mother liquor was concentrated under reduced pressure to give fraction B (40 g).

Fraction A was subjected to column chromatography (4x50 cm) packed with silica gel using $CHCl_3$ and $CHCl_3$-MeOH gradient for elution. The obtained subfractions were subjected to preparative TLC using silica gel plates and system II as a solvent system to obtain compounds **1** (50 mg) (R_f= 0.63), **2** (30 mg) (R_f= 0.31), **3** (10 mg) (R_f= 0.54), **4** (60 mg) (R_f= 0.38) **5** (55 mg) (R_f= 0.22) and system III to obtain compound **6** (70 mg) (R_f= 0.68).

Fraction B (40 g) was directly applied on the top of silica gel column (5x50 cm) and elution was done with $CHCl_3$, $CHCl_3$-MeOH graident to obtain compound **7** (60 mg) and compound **8** (50 mg).

Acid hydrolysis

Each of the isolated glucosides (5 mg) was dissolved in 5 ml N/2 H_2SO_4 mixed with an equal volume of water and refluxed for 2 hr. The aglycone was extracted with ether and subjected to TLC and spectral analysis. The acidic mother liquor of the hydrolysate containing the sugar moiety was neutralized, filtered and the filtrate was concentrated and spoted alongside with authentic sugar on whatmann No 1 filter paper using n-butanol-acetic acid-water (4:1:5) as solvent system.

Compound 1 (4α,5ß epoxyeupatolide): was obtained as needles, m.p. 135-137°C (Lit. 136-138°C),[4] IR ν ($CHCl_3$) cm^{-1}, 3450 (OH), 1760 (γ-lactone), 1625, 1430. HRMS m/z (rel. int.) 264.136 [M^+] (1.3) [Calc. for $C_{15}H_{20}O_4$ 264.136], 246 [$M-H_2O]^+$, (2.4), 161 (100).

Compound 2 (4α,5ß-epoxydesacetylovatifolin): was occured as colourless crystals, m.p. 158-160°C (Lit. 158-160°C),[4] IR ν ($CHCl_3$) cm^{-1}, 3440 (OH), 1750 (γ-lactone), 1625, 1620. HRMS m/z (rel. int.) 280.172 [M^+] (6) [Calc. for $C_{15}H_{20}O_5$ 280.175], 262 [$M-H_2O]^+$, (44), 245 [M^+-2H_2O] (64), 163 (100).

Compound 3 (5α-hydroxy dehydroleucodin): Colorless crystals, m.p. 171-3°C (LIt. 173°C).[10] IR ν ($CHCl_3$) cm^{-1}, 3580 (OH), 1775 (γ-lactone), 1695, 1645. HRMS m/z (rel. int.) 260.105 [M^+] (100) [Calc. for $C_{15}H_{16}O_4$ 260.104], 242 [$M-H_2O]^+$ (36), 227 [242-Me]$^+$. $[\alpha]_D^{20}$-8 ($CHCl_3$ C 0.1).

Compound 4 (14-hydroxy-2-oxyguaia-1 (10), 3-dien-5α, 11ßH-12,6α-olide): Gum IR ν ($CHCl_3$) cm^{-1}, 3450 (OH), 3050, 1780 (γ-lactone), 1685, 1620. HRMS m/z (rel. int.) 262 [M^+] (3) [Calc. for $C_{15}H_{18}O_4$ 262], 234 [M-CO]$^+$ (62), 219 [M-CO-Me]$^+$ (100). $[\alpha]_D^{20}$ +20 ($CHCl_3$ C 2.0).

Compound 5 2-oxo-8α,10ß-dihydroxyguai-3-en-1α,5α,6ß,11ßH-12,6 olide: Oil (Lit. oil),[12] IR ν ($CHCl_3$) cm^{-1}, 3400 (OH), 1775 (γ-lactone), 1685 (C=O), 1625. HRMS m/z (rel. int.) 280 [M^+] (3) [Calc. for $C_{15}H_{20}O_5$ 280], 262 [$M-H_2O]^+$ (100). $[\alpha]_D^{20}$ +32 ($CHCl_3$ C 3).

Compound 6: Yellow amorphous powder m.p. 347-349°C (Lit. 349°C),[6] UV (MeOH) λ_{max} nm 265, 338; + NaOMe 272, 292 sh, 390; + $AlCl_3$ 270, 298, 382; + $AlCl_3$/HCl 270, 381; + NaOAc 272, 375; + NaOAc/H_3BO_3 268, 338. MS m/z (rel. int.) 270.121 [Calc. $C_{15}H_{10}O_5$) 270.120] (100).

Compound 7: Yellow crystals m.p. 226°C (Lit. 226°C),[6] UV (MeOH) λ_{max} nm 270, 336; + NaOMe 270, 385; + $AlCl_3$ 275, 300, 385; + $AlCl_3$/HCl 280, 301, 345, 385; + NaOAc 270, 340; + NaOAc/H_3BO_3 270, 340. EIMS m/z (rel. int.) 270 [M^+] (100).

Compound 8: Yellow amorphous powder m.p. 210-212°C (Lit. 212°C),[16] UV (MeOH) λ_{max} nm 369, 325, 270 (sh), 255; + $AlCl_3$ 427, 357, 264; + $AlCl_3$/HCl 427, 357, 263; + NaOMe

413, 263; + NaOAc 409, 265; $NaOAc/H_3BO_3$ 365, 255. EIMS m/z (rel. int.) 330 (100), 315 (13), 301 (6); 287 (14), 259 (4), 244 (4), 165 (11), 153 (4).

All the ^1H and ^{13}C-NMR spectral data for compounds **1-8** are compiled in Tables 1-4.

RESULTS AND DISCUSSION

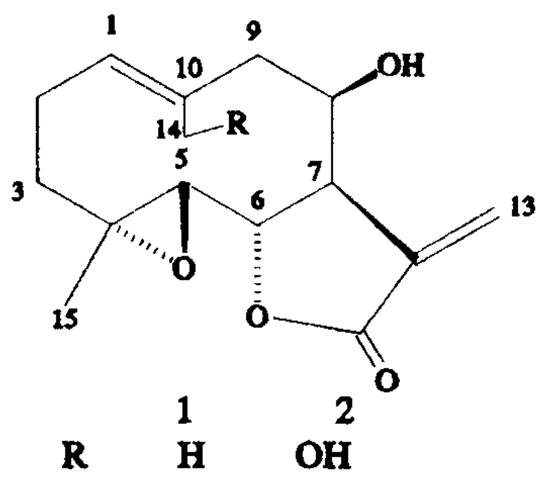
The aerial parts of *Inula britannica* (L.) afforded five known sesquiterpene lactones (two germacranoides 1 and 2 and three guaianolides 3, 4, 5) in addition to three known flavonoids.

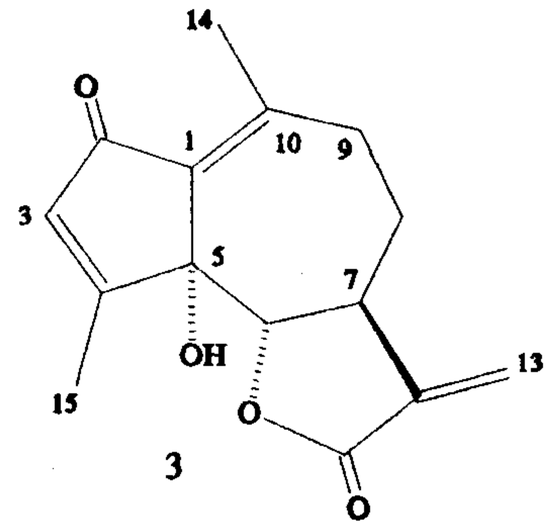
Compounds 1 & 2: The structures of these germacranolides were easily deduced from IR and ^1H-NMR data. In each case H-5 was shifted upfield in the region typical of epoxide signals. In the HRMS, the molecular ion peak (M^+) at m/z 264 in agreement with the molecular formula $C_{15}H_{20}O_4$ for compound 1 and at m/z 280 in agreement with molecular formula $C_{15}H_{20}O_5$ for compound 2. The spectral data of compounds 1 & 2 are in good agreement with those reported.[8,9]

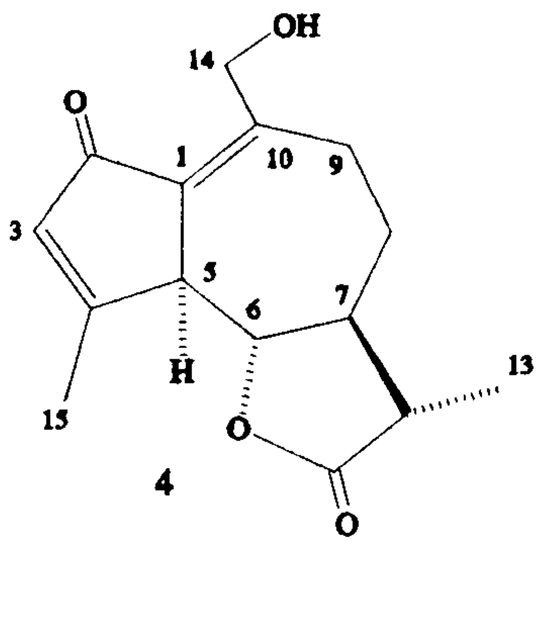
Guaianolides 3, 4 and 5: Exhibited IR characteristic for γ lactones. The mass spectrum showed the molecular ion peak (M^+) at m/z 260 in agreement with the molecular formula $C_{15}H_{16}O_4$ for compound 3. Peak at m/z 262 in agreement with molecular formual $C_{15}H_{18}O_4$ for compound 4 and at m/z 280 in agreement with molecular formula $C_{15}H_{20}O_5$ for compound 5. The above mentioned data for compounds 3, 4 & 5 are in good agreement with those reported.[9-13]

Compound 6: It showed physical, chemical, chromatographic characters and spectral data (UV, IR, ^1H-NMR, ^{13}C-NMR and MS) which compared favorably with those of apigenin.[14-17]

Compound 7: It is dihydroxy flavone glucoside as indicated from UV, ^1H-NMR and ^{13}C-NMR spectra (see Tables 3 & 4). Acid hydrolysis yielded aglycone which was identified as apigenin by direct comparison with authentic sample while the sugar moiety was characterized as glucose by co-chromatography with reference glucose. The position of the sugar was







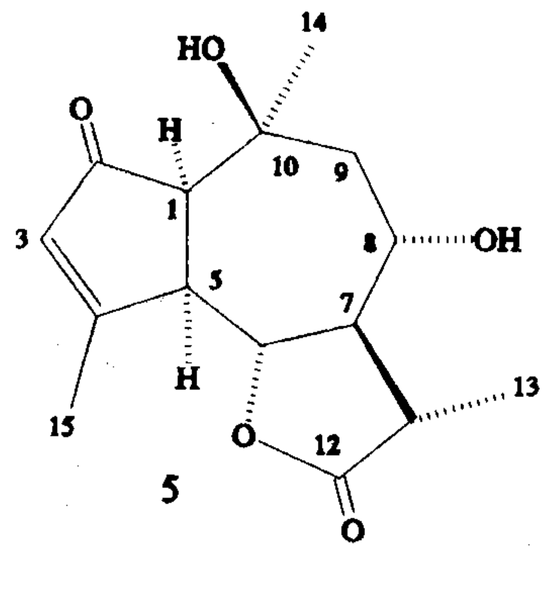


Table 1: ^{1}H-NMR data of **1-5** (200 MHz, $CDCl_3$).

	(1)	(2)	(3)	(4)	(5)
1	5,15, br. dd (13,2)	5.42, br. dd (13,2)	-	-	2.68, d (6.7)
2α	2.16, m	2.22, br. ddd (13,6,2)	-	-	
2ß	2.43, dddd	2.49, dddd	-	-	-
3α	1.21, ddd (13,6,1.5)	1.32, ddd (13,8.5,2)	6.13, q	6.19, brs	6.01, brs
3ß	2.11, ddd (13,5.5,1.5)	2.16, ddd (13,5.5,2)			
5	2.77, d (8.5)	2.82, d (8.5)	-	3.46, brd (10.5)	3.2, br dd (10,6.7)
6	4.57, dd (8.5,8.5)	4.55, dd (8.5,8.5)	3.91, d (10)	3.66, dd (10.5,10)	4.32, dd (10,10)
7	3.0, dddd	3.02, dddd	3.68, dddd	1.97, dddd	1.97, ddd (11.5,10,10)
8α	4.6, m	4.46, m	2.24, m	2.06, dddd	
8ß	-	-	1.49, m	1.38, dddd	4.1, ddd (10.5,10,3.5)
9α	2.59, dd (14,1)	2.76, dd (14,1)	2.95, brt	2.35, ddd (14.5,12.5,2.5)	1.72, ddd (14,10.5)
9ß	2.29, dd (14,5)	2.52, dd (14,5)	2.24, m	2.58, ddd (14.5,6,1.5)	2.09, dd (14,3.5)
11	-	-	-	2.26, dq (12.5,7)	2.6, dq (11.5,7)
13	6.33, d (3.5)	6.38, d (3.5)	6.17, d (3)	1.26, d (7)	1.45, d (7)
13'	5.62, d (3)	5.69, d (3)	5.45, d (2.5)	-	
14	1.84, br.s	4.45, br. d (12)	2.42, brs	4.60, brd (13)	1.58, s
14'	-	3.86, d (12)		4.50, brd (13)	
15	1.32 s	1.35, s	2.3, d (1.5)	2.35, brs	2.3, brs

J (paranthesis) in Hz.

Table 2: ^{13}C-NMR data of compounds **1, 2, 4, 5**, (50.3 MHz, $CDCl_3$).

Carbon	1	2	4	5
1	126.9 d	131.3 d	134.1 s	59.4 d
2	24.1 t	24.1 t	196.7 s	207.0 s
3	35.9 t	36.2 t	134.9 d	132.1 d
4	62.4 s	62.1 s	173.2 s	179.1 s
5	66.5 d	66.1 d	52.5 d	53.2 d
6	75.1 d	74.9 d	83.2 d	79.5 d
7	50.5 d	50.8 d	56.2 d	58.3 d
8	73.2 d	71.1 d	26.4 t	69.4 d
9	47.1 t	44.3 t	33.4 t	51.6 t
10	134.5 s	134.5 s	155.8 s	71.5 s
11	138.0 s	137.9 s	41.1 d	41.3 d
12	169.7 s	169.7 s	177.2 s	178.2 s
13	122.2 t	122.0 t	12.4 q	15.9 q
14	20.3 q	60.1 t	65.7 t	32.4 q
15	17.1 q	16.5 q	20.1 q	20.8 q

Table 3: ^{1}H-NMR spectral data of flavonoids **6-8** in DMSO-d_6.

Proton	6	7	8
H-3	7.51, s	7.5, s	-
H-6	6.32, d (2)	6.35, d (2)	6.44, d (2)
H-8	6.71, d (2)	6.70, d (2)	6.86, d (2)
H-2'	⎫ 7.7, dd (7,2)	⎫ 7.75, dd (7,2)	7.77, d (2.2)
H-6'	⎭	⎭	7.85, dd (8.5, 2.2)
H-3'	⎫ 6.8, dd (7,2)	⎫ 6.82, dd (7,2)	-
H-5'	⎭	⎭	7.15, d (J= 8.5)
C_5-OH	12.5 (s)	12.5 (s)	12.50 (s)
C_3-OH			9.7 (s)
H-1"		5.1, d (7)	5.02, d (7)
Sugar protons		3.4-4.1, m	3.4-4.1 m
-OCH_3			3.85 s
-OCH_3			3.84 s

determined from UV spectra of both glucoside and aglycone and was found to be at position No. 7. Accordingly compound 7 was identified as apigenin-7-O-ß-glucopyranoside[14,15].

Compound 8: Its UV absorptions indicated that it was a flavonol derivative and the ^{1}H and ^{13}C-NMR spectra suggested that it had a monosaccharide residue. The ^{1}H-NMR spectra showed the presence of a set of meta-coupled protons [δ 6.44 and 6.86 (J= 2 Hz)]. Three protons which showed an ABX spin system [δ 7.15 (J= 8.5 Hz), 7.77 (J= 2.2 Hz and 7.85 (J= 8.5, 2.2 Hz), two aromatic methoxyls (δ 3.84 and 3.85), two hydroxyl groups (9.7, 11.2)]. The above data and the EIMS spectral fragment at m/z 330 indicated that compound 8 is quercetin-3',4'-dimethyoxy-7-O-ß-glucopyranoside.[14-17]

Table 4: ^{13}C-NMR of flavonoids **6-8** (50.3 MHz, DMSO-d_6).

Proton	6	7	8
1			
2	164.1	164.5	146.9
3	102.8	103.2	136.5
4	181.8	181.9	176.3
5	161.5	161.5	160.3
6	98.8	99.6	98.7
7	163.5	163.1	162.7
8	94.0	95.1	94.5
9	157.3	156.9	155.6
10	103.7	105.4	104.4
1'	121.3	121.0	123.1
2'	128.4	128.5	111.4[a]
3'	116.1	116.5	148.3
4'	161.1	161.2	150.4
5'	116.0	116.5	110.8[a]
6'	128.4	128.5	121.6
1"		100.2	100.0
2"		73.3	73.0
3"		76.6[b]	76.4[b]
4"		69.8	69.4
5"		77.4[b]	77.2[b]
6"		60.9	60.5
MeO-3'			55.5
MeO-4'			55.5

a-b Interchangeable within the same compound.

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