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FLAVONOIDS FROM *ONOPORDON HETERACANTHUM* C.A. MEY

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في هذا البحث تم فصل سبعة فلافونويدات وهى ٣،٧،٦،٤'- تترامیثوكسى ٥،٣'- داى هيدروكسى فلافونول ، ٥،٧،٤'- ترای هيدروكسى ٦ ميثوكسى فلافون (هسبديولين) ، أبجنين ، ٥،٧،٣'،٤'- تتراهيدروكسى ٦ ميثوكسى فلافون ، ليوتيولين ، ليوتيولين ٧ -أ جلوكوزيد وليوتيولين ٧ -أ روتينوزيد بالإضافة إلى بيتاسيتوستيرول وبيتاسيتوستيرول جلوكوزيد. وقد تم التعرف على التركيب الكيميائى لهذه المركبات بإستخدام الطرق الطيفية المختلفة وقد وجد أن جميع هذه المركبات تم فصلها لأول مرة من النبات موضوع البحث وأن المركبات ٣،٧،٦،٤'- تترامیثوكسى ٥،٣'- داى هيدروكسى فلافونول ، ٥،٧،٣'،٤'- تتراهيدروكسى ٦ ميثوكسى فلافون واكاستين ٧ -أ روتينوزيد تم فصلهم لأول مرة من الفصيلة.

Seven flavonoids were isolated viz., 3,6,7,4'-tetramethoxy-5,3'-dihydroxy flavonol, 5,7,4'-trihydroxy-6-methoxy flavone (hispedulin), apigenin, 5,7,3',4'-tetrahydroxy-6-methoxy flavone, luteolin, luteolin-7-O-glucoside, acacetin-7-O-rutinoside, in addition to β-sitosterol and β-sitosterol glucoside from Onopordon heteracanthum. The structures of these compounds were established through the chemical and spectral studies. All the isolated compounds were reported for the first time from the titled plant, while the compounds 3,6,7,4'-tetramethoxy-5,3'-dihydroxy flavonol, 5,7,3'4'-tetrahydroxy-6-methoxy flavone and acacetin-7-O-rutinoside were isolated for the first time from the genus Onopordon.

INTRODUCTION

Onopordon heteracanthum C.A. Mey is a member of a small genus in the tribe Cynarea (Compositae).[1] The plants of the genus *Onopordon* have been employed traditionally for their antibacterial, heamostatic, and antihypertensive properties[2] and for treatment of face cancers since early times.[3] A pharmacological testing of *onopordopigin*, a sesquiterpene lactone which was isolatd from some plants belonging to the genus *Onopordon*, revealed anticancer activity.[4,5]

Flavonoids,[6-8] lignans,[6,9] and sesquiterpene lactones[10-14] being the most characteristic constituents of several species of the genus *Onopordon*.

EXPERIMENTAL

Plant material

The plant material was collected in March 2001 during the flowering stage, from El-Baha, Southwest Saudia Arabia. Identity was confirmed by Prof. Dr. A. Fayed, Prof. of Taxonomy, Faculty of Science, Assiut University. The aerial parts were dried and powdered.

Authentics

Authentic sugars were obtained from Merck, Darmstadt, Germany, β-sitosterol, β-sitosterol glucoside, luteolin and apigenin were obtained from the Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

Systems

I- Hexane - ethyl acetate (80:20)
II- Hexane - ethyl acetate (60:40)
III- Hexane - ethyl acetate (40:60)
IV- Chloroform - methanol (90:10)
V- Chloroform - methanol (85:15)
VI- Ethyl acetate - methanol (80:20)
VII- n-Butanol - acetone - formic acid - water (60:17:8:15)

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Equipments

All mps are determined using Sturat Scientific apparatus and uncorrected. Mass spectra were carried out on Jeol, JMS, 600H, NMR spectra were recorded at 400 and 100 MHz for ^{1}H and ^{13}C respectively in DMSO-d_6 and $CDCl_3$ using Jeol JNM-LA400. Silica gel for column (70-230 mesh), TLC using precoated silica gel sheets (E-Merck).

Flavonoidal spots were visualized by their fluorescence at 254 nm under UV lamp or by spraying with $AlCl_3$. β-sitosterol spots was visualized by spraying with anisaldehyde solution and heating at 105° for 10 minutes.

Extraction and isolation

The air dried aerial parts of the plant (1.0 kg) were defatted with n-hexane (4x1.5 L) and successively extractd with chloroform (5x1.5 L) and ethyl acetate (4x1.5 L) to give 50 and 20 g of residue, respectively. Part of the chloroform extract residue (20 g) was chromatographed on a silica gel column using hexane and hexane - ethyl acetate, gradient method to give six pure compounds.

Five grams of the ethyl acetate fraction (20 g) was chromatographed on a silica gel column using chloroform - methanol gradient to give three pure compounds.

Acid hydrolysis

15 mg of each compound was refluxed with 2N HCl-EtOH (1:1, 15 ml) on a steam bath for 4 hours. The reaction mixture was diluted with water and extracted with $CHCl_3$ (3x250 ml). The chloroformic extract was concentrated under reduced pressure. Each aglycone was purified by recrystallization from $CHCl_3$ containing few drops of methanol. Each recovered aglycone part was identified by direct comparison with authentic samples on TLC using solvent systems II and III for flavonoid aglycones and system I for β-sitosterol. The aqueous layer was neutralized and the sugar was identified by TLC (using a solvent system VII) and comparison with authentic samples.

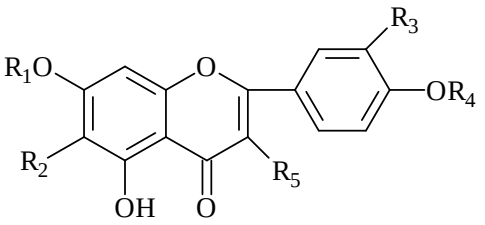
RESULTS AND DISCUSSION

The aerial parts of *Onopordon heteracanthum* afforded four flavones, one flavonol, two flavone glycosides, β-sitosterol and β-sitosterol glucoside.

Compound 1: Eluted with hexane - ethyl acetate (9:1) as white fine needles (MeOH), 84 mg, m.p 135-137°, R_f 0.52 (solvent system I). This compound was identified as β-sitosterol by direct authentication (m.m.p and co-chromatography using system I).

Compound 2: Eluted with hexane - ethyl acetate (85:15) as yellow powder (MeOH), 50 mg, R_f 0.50 (solvent system II), m.p 228-230°. The MS (rel. int.%): 374 [M^+] base, 359 (79), 356 (50), 331 (69), 181 (6), 178 (13), 153 (10) and 151 (9). The UV spectral analysis of compounds **2** in methanol showed absorption at λ_{max} 346 nm which characteristic for 3-blocked flavonol or flavone. The shift in $AlCl_3$, $AlCl_3$/HCl (band I) and NaOAc (band II) in Table 1 indicated the absence of orthodihydroxy group in ring B (3',4'-orthodihydroxy group) and absence of free OH at C-7 respectively.[15] The ^{1}H-NMR data (Table 2) revealed doublets at δ 7.7 (J= 1.7 Hz) for H-2', at 7.05 (J= 8.5 Hz) assigned for H-5' and at 7.6 (dd, J= 8.5, 1.7) for H-6', a singlet at 6.05 (s) for H-8 and four singlets at δ 3.85, 3.92, 3.95 and 3.98 (s) assigned for four methoxy groups at C-4', C-7, C-6 and C-3 respectively. The MS displayed molecular ion peak at m/z 374 consistent with the molecular formula $C_{19}H_{18}O_8$ and in accordance with flavonols with four methoxy and two hydroxy groups. The ^{13}C-NMR (Table 3) showed that a methoxy group (downfield at δ 60.1) was flanked by ortho-oxygenated carbons.[16] The other signal (downfield at δ 60.8) was attributed to a methoxy group at C-3 flavonol.[11] The rest of the signals were in accord to the flavonol 3,6,7,4'-tetramethoxy-5,3'-dihydroxy flavonol. The already mentioned data indicated that compound **2** was 3,6,7,4'-tetramethoxy-5,3'-dihydroxy flavonol.

Compound 3: Eluted with hexane - ethyl acetate (80:20) as yellow needles (MeOH), 40 mg, R_f 0.46 (solvent system II), m.p 264-267°. The MS (rel. int.%): 300 [M^+] (10), other characteristic fragments at 285 (67), 272 (11), 269 (8), 257 (57), 183 (10), 153 (11), 139 (19), 121 (13), 118 (33) and 69 (68). Its UV absorption data (Table 1) with different ionizing



Compd.	R_1	R_2	R_3	R_4	R_5
2	Me	OMe	OH	Me	OMe
3	H	OMe	H	H	H
4	H	H	H	H	H
5	H	OMe	OH	H	H
6	H	H	OH	H	H

Table 1: UV spectrald ata of compounds **2, 3, 4, 5, 6, 8** and **9**.

Comp. No.	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
2	253 273 346	269 363	266 377	266 366	254 366	260 344
3	273 333	274 325 (sh) 390	285 358	286 351	286 350	277 339
4	262 337	270 290 (sh) 392	269 298 (sh) 380	270 380	272 374	267 337
5	274 343	265 400	275 423	286 365	279 345	266 371
6	253 267 350	266 330 401	273 301 435	275 364 388	270 385	260 301 380
8	270 295 352	271 300 400	270 300 (sh) 325 (sh) 430	271 290 (sh) 360 (sh) 388	264 350 (sh) 412	265 370
9	269 347	282 376	276 299 (sh) 348 (sh) 382	276 299 (sh) 338 (sh) 383	268 324	268 325

Table 2: ^{1}H-NMR spectral data of compounds **2, 3** and **5** in DMSO-d_6.

Comp. No.	H-3	H-8	H-2'	H-3'	H-5'	H-6'	OCH_3 group
2*	-	6.50 s	7.7 (d, J= 1.7 Hz)	-	7.05 (d, J= 8.5 Hz)	7.6 (dd, J= 8.5, 1.7 Hz)	3.85, 3.92, 3.95, 3.98 (s)
3	6.5 (s)	6.7 (s)	7.9 (d, J= 8.8 Hz)	6.8 (d, J= 8.8 Hz)	6.8 (d, J= 8.8 Hz)	7.9 (d, J= 8.8 Hz)	3.73 (s)
5	6.5 (s)	6.7 (s)	7.3 (br.s)		6.94 (d, J= 8.5 Hz)	7.4 (d, J= 8.5 Hz)	3.73 (s)

*In $CDCl_3$.

Table 3: ^{13}C-NMR spectral data of compounds **2, 3** and **5** (DMSO-d_6 for compounds **3** and **5**; $CDCl_3$ for compound **2**).

Carbon no.	**2***	**3**	**5**
2	155.9	163.8	163.9
3	138.6	102.3	102.4
4	178.8	182.0	182.0
5	152.7	152.7	152.8
6	132.2	131.3	131.3
7	158.7	157.2	157.3
8	90.3	94.1	94.1
9	152.2	152.3	152.4
10	106.5	104.0	104.0
1'	122.5	121.0	121.5
2'	110.8	128.4	113.3
3'	146.3	115.9	145.7
4'	148.3	161.0	149.7
5'	114.5	115.9	116.0
6'	122.4	128.4	118.9
OMe	60.8	59.9	59.9
	60.1		
	56.3		
	56.1		

*In $CDCl_3$.

and complexing reagent showed that the compound **3** contains free OH group at C-7 (NaOAc shift, band II), and a free 4'-hydroxy group (bathochromic shift of band I in NaOMe with increase in intensity) and no ortho-dihydroxy group in A ring ($AlCl_3$, $AlCl_3$/HCl). The ^{1}H-NMR spectrum (Table 2) showed the presence of signals at δ 6.7 (1H, H-8) for A-ring and 6.5 (1H, H-3) for the proton γ-pyrone ring, at δ 6.8 (2H, H-3',5'), 7.9 (2H, H-2',6') for the protons of B-ring and the singlet at δ 3.73 (3H, OCH_3) was attributed to the O-methyl group at C-6'. The mass spectrum of **3** showed a peak at m/z 300 corresponding to the molecular formula $C_{16}H_{12}O_6$ (DEPt, ^{13}C-NMR and MS). The ^{13}C-NMR (Table 3) showed that the methoxy group (downfield at δ 59.9) was flanked by ortho-oxygenated carbons (i.e. 5,7-dihydroxy-6-methoxy flavone).[16] The structure was moreover supported by the close resemblance of the physical and spectral data (UV, ^{1}H-NMR and ^{13}C-NMR) with those reported for 5,7,4'-trihydroxy-6-methoxy flavone (hispedulin).[17]

Compound 4: Eluted with hexane - ethyl acetate (78:22) as yellow amorphous powder (MeOH), 15 mg, R_f 0.40 (solvent system II), m.p 343-345°. The MS (rel. int.%): 270 [M^+] (23), 269 (87), 241 (70), 240 (50), 153 (77), 149 (45), 121 (74), 114 (34) and 69 (70). UV (Table 1), m.p, m.m.p and co-chromatography with authentic sample indicated that compound **4** is apigenin.

Compound 5: Eluted with hexane - ethyl acetate (60:40) as yellow needles (MeOH), 50 mg, R_f 0.28 (solvent system II), m.p 259-262°. The MS (rel. int.%): 316 [M^+] (56), 300 (40), 297 (28), 272 (49), 197 (12), 134 (60) and 69 (100). The UV spectra (Table 1) in different shift reagents indicated the presence of free OH at C-7 (NaOAc), 3',4'-ortho-dihydroxy group in ring-B ($AlCl_3$, $AlCl_3$/HCl and NaOAc/boric acid spectra) and 4'-OH (NaOMe, bathochromic shift with increase in intensity). The ^{1}H-NMR (Table 2) showed signal at δ 6.5 (1H, s, H-3), signal for A-ring proton at δ 6.7 (1H, s, H-8), signals for B-ring protons at δ 6.94 (1H, d, H-5'), 7.4 (1H, d, H-6'), 7.3 (1H, br.s, H-2') and 3.73 (3H, s, OCH_3 at C-6). The mass spectrum showed a peak at m/z 316 corresponding to the molecular formula $C_{16}H_{12}O_7$. The ^{13}C-NMR (Table 3) showed a characteristic downfield shift signal at δ 59.9 for methoxy group at C-6 between two ortho-oxygenated carbon (i.e. 5,7-dihydroxy-6-methoxy flavone). The downfield shift of C-4 at δ 182.0 as in compound **3** indicated the presence of hydroxy at C-5 in flavone.[16]

The above mentioned data suggest the flavonoid **5** is 5,7,3',4'-tetrahydroxy-6-methoxy flavone. The structure of compound **5** was moreover supported by the close resemblance of the ^{13}C-NMR spectral data with those reported for 5,7,3',4'-tetrahydroxy-6-methoxy flavone.[18]

Compound 6: Eluted with hexane - ethyl acetate (10:90) as yellow amorphous powder (MeOH), 18 mg, R_f 0.23 (solvent system III), m.p 328-330°. MS m/z (rel. int.%): 286 (100), 273 (22), 258 (18), 229 (8) and 203 (6). UV (Table 1), m.p, m.m.p and co-chromatography with authentic sample indicated that compound **6** is luelolin.

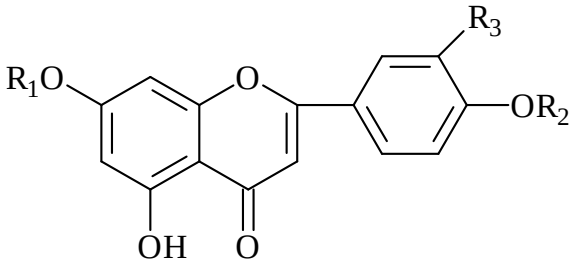
Compound 7: Eluted with chloroform - methanol (90:10) as white powder, 400 mg, R_f 0.44 (solvent system IV), m.p 278-279°. It was identified as β-sitosterol glucoside by direct authentication (m.p, m.m.p, co-chromatography) as well as the aglycone β-sitosterol upon acid hydrolysis (see experimental part).

Compound 8: Eluted with chloroform - methanol (85:15) as yellow amorphous powder (MeOH), 150 mg, R_f 0.58 (solvent system V), m.p 255-258°. Its UV data (Table 1) indicated that compound **8** is a flavone with 3',4'-ortho-dihydroxy group ($AlCl_3$, $AlCl_3$/HCl and NaOAc/H_3BO_3 shift) and absence of OH group at C-7 (NaOAc shift). The ^{1}H-NMR spectral data (Table 4) showed signals for γ-pyrone ring at δ 6.7 (s, H-3), for A-ring at δ 6.4 (d, H-6), 6.8 (d, H-8), for B-ring at δ 7.0 (d, H-5'), 7.5 (dd, H-6'), 7.4 (d, H-2'). Anomeric proton of glucose appeared at δ 5.1 (1H, d). Acid hydrolysis (see experimental) gave aglycone whose data are identical for luteolin and the sugar was found to be glucose (co-chromatography with authentic sample).

From the previous data and comparing those data with the published data for luteolin-7-O-glucoside,[15,19] so compound **8** was identified as luteolin-7-O-glucoside. The above data was confirmed by ^{13}C-NMR data (Table 5).

Compound 9: Eluted with chloroform - methanol (80:20) as yellow amorphous powder (MeOH), 90 mg, m.p 238-240°, R_f 0.50 (Solvent system VI). The UV data (Table 1) showed that compound **9** contains no free OH at C-4', C-7 and absence of ortho-dihydroxy group from the NaOMe, NaOAc and $AlCl_3$, $AlCl_3$/HCl respectively. The ^{1}H-NMR spectrum (Table 4) showed aromatic protons of C-2' and of C-6' appeared at δ 8.03 (d, 2H), protons of C-3' and C-5' at δ 7.1 (d, 2H), proton of C-3 at δ 6.7 (s, 1H), proton at δ 6.4 (1H, br.s) for C-6 and at δ 6.9 (1H, br.s) for C-8, anomeric proton of glucose appeared at δ 5.1 (1H, d) and that for rhamnose at δ 4.5 (1H, br.s) and CH_3 for rhamnose at δ 1.07 (1H, d). The methoxy protons appeared at δ 3.84 (3H, s). Acid hydrolysis (see experimental) gave aglycone, whose identical to acacetin (co-chromatography, m.p and m.m.p with authenic sample), and the sugars by using TLC chromatography gave spots of glucose and rhamnose when compared with authentic sugars.

From the previous data and comparing with the published data[20] the compound **9** was suggested to be acacetin-7-O-rutinoside which confirmed by ^{13}C-NMR data (Table 5).



Compd.	R_1	R_2	R_3
8	glucose	H	OH
9	glucose-rhamnose	CH_3	H

Table 4: ^{1}H-NMR spectral data of compounds **8** and **9** in DMSO-d_6.

Comp. No.	H-3	H-6	H-8	H-2' (comp. **8**)	H-5' (comp. **8**) H-3',5' (comp. **9**)	H-6' (comp. **8**) H-2',6' (comp. **9**)	OCH_3 group
8	6.7 (s)	6.4 (d, J= 1.9 Hz)	6.8 (d, J= 1.9 Hz)	7.4 (d, J= 2.1 Hz)	7.0 (d, J= 8.5 Hz)	7.5 (dd, J= 8.5, 2.1 Hz)	-
9	6.7 (s)	6.4 (br.s)	6.9 (br.s)	-	7.1 (d, J= 8.5 Hz)	8.03 (d, J= 8.5 Hz)	3.84 (s)

The sugar protons:

a) Compound **8** shows signal at δ 5.1 (d, J= 7.3 Hz) for anomeric proton of glucose.

b) Ccompound **9** shows signal at δ 5.06 (d, J= 7.08 Hz) for anomeric proton of glucose. Signal at δ 4.5 (br.s) for anomeric proton of rhamnose and signal at δ 1.07 (d, J= 6.1 Hz) assigned for CH_3 of rhamnose.

Table 5: ^{13}C-NMR spectral data of compounds **8** and **9** (DMSO-d_6).

Carbon no.	**8**	**9**
2	164.5	164.0
3	103.2	103.8
4	181.9	181.4
5	161.1	161.1
6	99.9	99.9
7	162.9	162.9
8	94.7	94.8
9	156.9	157.0
10	105.4	105.4
1'	121.4	122.6
2'	113.5	114.7
3'	145.8	128.5
4'	149.9	162.4
5'	116.0	128.5
6'	119.2	114.7
OMe	-	55.6
1"	99.5	99.7
2"	73.1	73.1
3"	76.4	76.3
4"	69.5	69.6
5"	77.2	77.1
6"	60.6	68.3
1"'	-	100.5
2"'	-	70.3
3"'	-	70.7
4"'	-	72.0
5"'	-	75.7
6"'	-	17.8

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