Bull. Pharm. Sci., Assiut University, Vol. 21, Part 2, December 1998, pp. 103-108.

FLAVONOIDS FROM *PULICARIA ARABICA* (L.) CASS.

Mahmoud A. Ramadan

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

تم فى هذا البحث فصل والتعرف على تسع مركبات فينولية تفصل لأول مرة من النبات موضوع البحث وهى ٢-هيدروكسى-٦،٤-داى ميثوكسى اسيتوفينون ، ٧،٣-داى ميثوكسى كامبفيرول ، ٣-ميثوكسى كامبفيرول ، كامبفيرول ، داى هيدرو كامبفيرول ، ٧،٣-داى ميثوكسى كورسيتين ، ٣-ميثوكسى كورسيتين ، كورسيتين ، داى هيدروكورسيتين. وقد تم التعرف على هذه المركبات بإستخدام الطرق الطيفية المختلفة.

From the chloroformic extract of the aerial parts of Pulicaria arabica (L.) Cass. eight flavonoids were isolated. Their structures were established by spectroscopic methods (UV, MS and NMR spectroscopy) and proved to be 3,7-dimethoxy kaempferol, 3-methoxy kaempferol, kaempferol, dihydro kaempferol, 3, 7-dimethoxy quercetin, 3-methoxy quercetin, quercetin and dihydro quercetin. In addition, an acetophenone was also isolated.

INTRODUCTION

The genus *Pulicaria* (Family Asteraceae) is reported to have several folkloric medicinal uses in Egypt.[1] It is represented in Egypt by six species one of which *Pulicaria arabica* (L.) Cass.[2] Plants of the genus have been subjected to many chemical investigations notably involving flavonoids[3-7] and terpenoids.[2,8,9] Melek *et al.*[7] reported the isolation of isorhamnetin, 5-hydroxy-3, 6,7,3´,4´-pentamethoxyflavone, 4´-hydroxy-3,5,6,7,3´-pentamethoxyflavone and 3,5,6,7,3´,4´-hexamethoxyflavone from the total herb of the title plant.

In the present work, we report the isolation of nine phenolic compounds reported for the first time from *Pulicaria arabica* (L.) Cass.

EXPERIMENTAL

General experimental procedures

Mps are uncorrected, UV spectra are measured in MeOH and different ionizing and complexing agents using Unicam 1750 spectrophotometer. ^{1}H-NMR spectra were run in DMSO-d_6 and $CDCl_3$ at 300 MHz using Varian XL. 300, using TMS as internal standard. EIMS were recorded at 70 ev. Column chromatography: Silica gel (E. Merck, 6.3-20 μ) and pre-packed column (LiChroprep. E. Merck) under pressure 2-3 bar, were used for fine separation of the compounds. TLC was performed on Silica gel G (E. Merck) activated layers using $CHCl_3$-MeOH (90:10) (system I), $CHCl_3$-MeOH (85:15) (system II) and $CHCl_3$:MeOH:water (35:7:2) (system III). Spots were detected under UV before and after exposure to ammonia.

Plant material

The aerial parts of *Pulicaria arabica* (L.) Cass. was collected from Ismailia-EL-Arish road in April 1995. The identity was confirmed by Dr. S. EL-Naggar Department of Botany, Faculty of Science, Assiut University.

Isolation and characterization of compounds

4 Kg of the air-dried aerial parts of *Pulicaria arabica* (L.)Cass. was extracted with ethanol (95%) by maceration and percolation. The alcoholic extract was concentrated under reduced pressure to syrupy consistency, defatted with petroleum ether, then successively extracted with $CHCl_3$,EtOAc and n-butanol.

The concentrated $CHCl_3$ extract revealed the presence of at least 12 phenolic spots (spraying with $FeCl_3$ and 5% KOH).

The $CHCl_3$ extract (20 g) was fractionated on a silica gel column (120x4 cm) using $CHCl_3$:

Received in 12/5/1998 & Accepted in 3/8/1998

MeOH gradients. Fractions eluted with $CHCl_3$: MeOH (99:1) afforded two components which were separated by repeated CC to afford compounds **1** and **2**.

Fractions eluted with $CHCl_3$: MeOH (97.5:2.5) afforded a mixture of three components and were separated on prepacked silica gel columns (LiChroprep) using $CHCl_3$: MeOH (98:2) as solvent system where compounds **3**, **4** and **5** were isolated. The fractions eluted with $CHCl_3$: MeOH (95:5) afforded a mixture of four compounds and which was separated on prepacked columns to give compounds **6**, **7**, **8** and **9**, respectively.

Compound 1: needles from MeOH, m.p 83-84°C. IR ν_{max} cm^{-1}: 3350 (OH, chelated), 1640 (CO, chelated). ^{1}H-NMR (DMSO): δ 13.8 (1H,s, OH at C-2), 6.07 (1H, d, J= 2.3 Hz, H-5), 6.11 (1H, d, J= 2.2 Hz, H-3), 3.8 (3H, s,OCH_3-4), 3.84 (3H, s, OCH_3-6), 2.45 (3H, s, OC.$\underline{CH_3}$). EIMS, m/z (rel. int. %): 196 (M^+) (100), 181 (80), 167(10), 153(5).

Compound 2: Yellowish-brown amorphous powder, m.p 236-238° C, R_f= 0.61 (system I). EIMS, m/z (rel. int. %): 314 (100), 313 (85), 296 (15), 285(16), 283 (10), 271(30).

Compound 3: Yellowish-brown amorphous powder, m.p 247-248° C, R_f= 0.56 (system I). EIMS, m/z (rel. int. %): 300 (68), 299 (52), 288 (10), 271 (10), 269 (6) and 257(31).

Compound 4: Yellow needles (MeOH), m.p 284-286°C, R_f= 0.53 (system I). EIMS, m/z (rel. int. %): 286 (70), 258 (51), 153 (7), 152 (6), 121 (10).

Compound 5: White needles, m.p 226-228°C, R_f= 0.48 (system I). EIMS, m/z (rel. int. %): 288 (21), 153 (100), 136 (38).

Compound 6: Brown amorphous powder, m.p 236-238°C, R_f= 0.46 (system II). EIMS, m/z (rel. int. %): 330 (M^+), 329 (90), 312 (16), 301 (12), 299 (6), 287 (40).

Compound 7: Yellow amorphous powder, m.p 213-15°C, R_f= 0.38 (system II).

Compound 8: Yellow needles, m.p 314-16°C, R_f= 0.32 (system II). EIMS, m/z (rel. int. %): 302 (100), 286 (85), 274 (15), 273 (16), 228 (10), 152 (4), 153 (12), 138 (15), 137 (32), 122 (16).

Compound 9: Pale white needles, m.p 232-234°C, R_f= 0.50 (system III). EIMS, m/z (rel. int. %): 304 (52), 276 (65), 154 (13), 153 (100), 152 (3), 150 (28).

The UV and NMR data of compounds **2-9** are listed in Tables 1 and 2, respectively.

RESULTS AND DISCUSSION

Compound 1: was obtained as needles (MeOH) and melted at 83-85°C. It has the molecular formula $C_{10}H_{12}O_4$ based on EIMS and NMR spectral data. Its IR spectrum showed absorptions at 3350 and 1640 cm^{-1} for both chelated OH and CO groups, respectively. The ^{1}H-NMR (DMSO) spectrum supported this finding by showing a singlet at δ 13.8 for the strongly chelated proton of the OH group at C-2, and a singlet at 2.45 for OCO-$\underline{CH_3}$. It also showed two doublets of two meta coupled aromatic protons at δ 6.07 and 6.11 (J= 2.2 Hz) for H-5 and H-3, respectively. In addition, the spectrum showed other three singlets at δ 3.8 (3H), 3.84 (3H) and 2.45 (3H) assigned for OCH_3-4, OCH_3-6 and OC-$\underline{CH_3}$, respectively. The determination of the structure of **1** was accomplished only through NOE. The position of OCH_3 at C-6 is deduced from NOE between the methoxyl singlet at δ 3.8 and the singlet at δ 2.45. Accordingly the structure of compound **1** was deduced as 2-hydroxy-4,6-dimethoxy acetophenone previously isolated from *Pulicaria undulata*.[10]

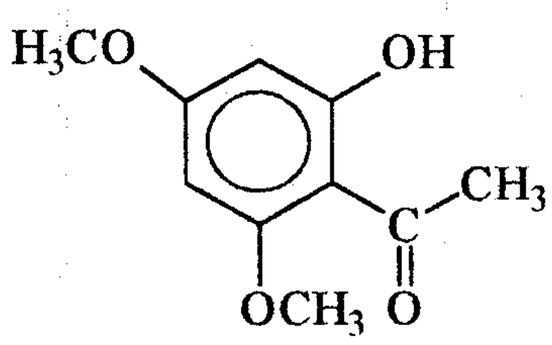
Compound 2: was a flavonol as indicated from its UV absorption (Table 1) (band I in MeOH at 350 nm), and possessed a 4´-hydroxyl group (band I NaOMe bathochromic shift of 48 nm

Table 1: UV Spectral data of the isolated flavonoids.

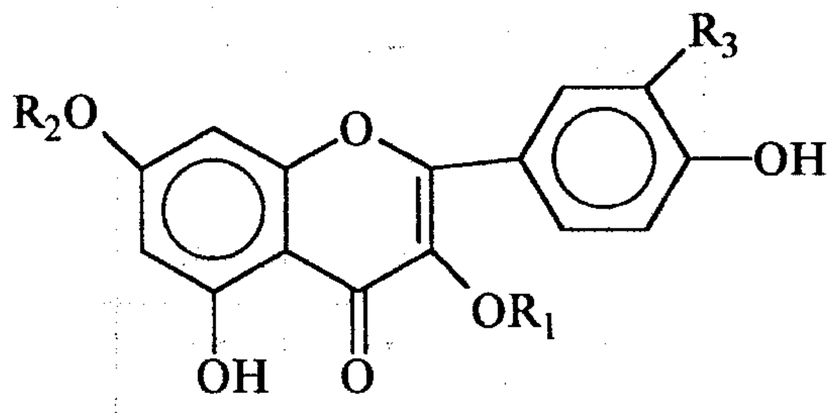
Reagent compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$+HCl	NaOAc	NaOAc+H_3BO_3
2	266 294 348	256 266 296 396	275 302 350 398	275 299 346 397	266 388	265 350
3	286 299 349	275 325 396	277 305 350 399	227 305 347 399	277 310 389	268 303 351
4	267 255 367	270 414	270 305 426	270 305 390	276 330 390	269 320 375
5	293 328	245 326	273 317 366	278 314 364	279 329	295 335
6	256 266 294 359	267 298 407	276 299 332 441	267 298 362 402	262 296 362 	259 380
7	257 270 295 357	272 327 407	277 305 440	271 305 365 406	272 334 385 	261 295 377
8	256 269 371	273 255 412(dec)	272 303 363 438	266 304 360 391	273 328 396	261 328 392
9	291 328	246 326(dec)	280 310 375	312 375	290 329	292 337

Table 2: ^{1}H-NMR data of the isolated compounds.

Compd.	H-2	H-3	H-2´	H-6´	H-3´	H-5´	H-8	H-6	3-OMe	7-OMe
2	--	--	7.95, dd (8.5, 2)	7.95, dd (8.5, 2)	6.85, dd (8.5, 2)	6.85, dd (8.5, 2)	6.43, d (2.5)	6.15, d (2.5)	3.83, s	3.83, s
3	--	--	8.04, dd (9, 2.5)	8.04, dd (9, 2.5)	6.95, dd (9, 2.5)	6.95, dd (9, 2.5)	6.67, d (2.5)	6.25, d (2.5)	3.83, s	--
4	--	--	8.2, dd (9, 2.2)	9.2, dd (9, 2.2)	6.95, dd (9, 2.2)	6.95, dd (9, 2.2)	6.55, d (2)	6.4, d (2)	--	--
5	4.95, d (11.5)	4.51, d (11.5)	7.35, dd (8.8, 2.2)	7.35, dd (8.8, 2.2)	6.80, dd (8.8, 2.2)	6.80, dd (8.8, 2.2)	6.05, d (2.2)	6.01, d (2.2)	--	--
6	--	--	7.66, d (2.0)	7.43, dd (8.5, 2)	--	6.95, d (8.5)	6.55, d (2.5)	6.30, d (2.5)	3.84, s	3.78, s
7	--	--	7.73, d (2)	7.58, dd (8.5, 2)	--	7.12, d (8.5)	6.40, d (2.5)	6.24, d (2.5)	3.82, s	
8	--	--	7.83, d (2)	7.80, dd (2,8)	--	7.18, d (8)	7.48, d (2)	6.62, d (2)	--	--
9	4.93, d (11)	4.47, d (11)	6.82, m	7.00, m	--	6.82, m	5.90, d (2.5)	5.85, d (2.5)	--	--



Compound 1



	R_1	R_2	R_3
Compound 2	Me	Me	H
Compound 3	Me	H	H
Compound 4	H	H	H
Compound 6	Me	Me	OH
Compound 7	Me	H	OH
Compound 8	H	H	OH

and increase in intensity compared with band I in MeOH.[11] The MS of 2 exhibited a molecular ion peak at m/z 314 in accordance with a flavonol containing two hydroxyl and two methoxyl groups. The ^{1}H-NMR spectrum confirmed the presence of these groups and exhibited signals for six aromatic protons characteristic for kaempferol (Table 2). The compound has purple fluorescent spot on paper under UV light and turned yellow with ammonia indicating the presence of free 5-OH[11] and substitution in 3-position by a methoxyl group, while the second methoxyl was at C-7(no shift with NaOAc reagent). From the abovementioned data, compound **2** could be identified as Kaempferol 3,7-dimethyl ether.[12]

Compound 3: The mass spectrum of **3** exhibited a molecular ion at m/z 300 in accordance with a flavonol containing three hydroxyls and one methoxyl group and exhibited signals for six aromatic protons; two sets of two proton doublets (J= 8.5 Hz) at δ 7.88 and 6.88 for a typical kaempferol type B-ring[1] and two sets of one-proton doublets (J= 2.5 Hz) at δ 6.44 and 6.73 typical for protons at C-6 and C-8 respectively.[11] The presence of a hydroxyl group at C-4´ was confirmed by a band I bathochromic shift of 47 nm with increased intensity in NaOMe relative to band I in MeOH. The presence of OH at C-7 was confirmed by band II bathochromic shift of 11 nm. The presence of a hydroxyl group at C-5 was evident since the compound appears as a purple spot on a paper chromatogram when viewed in UV light. These results indicated that the only position available for the methoxyl group is at 3. So compound **3** was identified as kaempferol 3-methyl ether.

Compounds 4 and 8: were identified as kaempferol and quercetin, respectively, by studying their physical, chemical and UV spectral data with different ionizing and complexing agents (Table 1) as well as ^{1}H-NMR spectra[11] (Table 2).

Comparison of the ^{1}H-NMR data of **4** with those of **5** (Table 2), revealed that compound **5** is the 2,3-dihydroderivative of **4**, based on the appearance of new resonances at δ 4.95 (1H, d, J= 11.5 Hz) and 4.51 (1H, d, J= 11.5 Hz) for the protons H-2 and H-3, respectively. The other resonances in the spectrum are comparable with those of **4** (Table 2). Also its mass spectrum showed a molecular ion at m/z 288 exceeding compound **4** with 2 mass units. Therefore, compound **5** was identified as dihydro kaempferol.[11]

Compound 6: was obtained as yellow amorphous powder. Its UV spectral data (Table 1) indicated that it is a flavone with free hydroxyl groups at 5,4´ and ortho dihydroxy function in ring B. ^{1}H-NMR data (Table 2) indicated that it contains two methoxyl groups at δ 3.78 and 3.84 and revealed the five aromatic protons of quercetin type flavonoids. Following the scheme of Voirin[14] for identification of 5-hydroxy-and 5-hydroxy 3-methoxyflavones with mono, di- and tri-substituted B ring,[14] it could be concluded that compound **6** is 3,7-dimethoxy quercetin.[12]

Compound 7: was obtained as a yellow amorphous powder melted at 213-215°C. The NMR spectrum confirmed the presence of one methoxyl group (δ 3.78). In addition, NMR signals were observed for five aromatic protons in accord with a quercetin type flavonol (δ 7.73, 7.12 and 7.58 for the B-ring and 6.40 and 6.24 for the A-ring protons). The presence of a hydroxyl group at C-5 was evident since the compound appears 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 (+15 nm) of band II in NaOAc relative to band II in methanol.[1] The bathochromic shift in band I (+20 nm) and (+ 4 nm) in band II with $NaOAc/H_3BO_3$, indicates the presence of free orthodihydroxy groups in ring B. So compound **7** was identified as quercetin 3-methyl ether.

Compound 9: mp 231-233°C, gave a violet colour with aqueous $FeCl_3$ and yellow colour with alkali suggesting a phenolic compound with a chromone moiety. The UV_{max} at 291 and 332 nm were closely related to dihydroflavonol.[15] A bathochromic shift (27 nm) on addition of $AlCl_3$ indicated the presence of a chelated hydroxyl group peri to carbonyl. The ^{1}H-NMR spectrum

revealed a pair of doublets at δ 4.93 and 4.47 typical of the AB system of the vicinal protons at C-2 and C-3 of 3-hydroxy flavanone.[16] The remaining features of ^{1}H-NMR spectrum defined 5,7- and 3´,4´-dihydroxylations in rings A and B, respectively. The above-mentioned data confirmed its identity as taxifolin (dihydroquercetin).[11,17,18]

REFERENCES

1- J. M. Watt and M. G. Breyer-Brandwijk: "The Medicinal and Poisonous Plants of Southern and Eastern Africa", 2nd Ed. E & S Lavingstone. Ltd, Edinburgh. London (1962).
2- V. Tackholm: "Students, Flora of Egypt", 2nd Ed. Cairo University, 562 (1974).
3- D. W. Bishay, C. S. Gomaa and M. H. Assaf, Bull. Pharm. Sci., Assiut University, 5, 65-71 (1982).
4- J. O. Pares, S. Oksuz, A. Ulabelen and T. J. Mabry, Phytochem., 20, 2057 (1981).
5- S. I. El-Negoumy, R. M. A. Mansour and N. A. M. Saleh, Phytochem., 21, 953-54 (1982).
6- M. Abdel-Mogib, A. M. Dawidar, M. A. Metwally and M. Abou-EL-Zahab, Pharmazie, 44 (11), 801 (1989).
7- F. R. Melek, M. A. El-Ansari, A. Hassan, A. Regaila, A. A. Ahmed and T. J. Mabry: Rev. Latinoamer. Qufn. 19/3, 119-120 (1988).
8- F. Bohlmann, K. H. Knoll and N. A. ElEmary, Phytochem. 18, 1231 (1979).
9- S. Hafez, T. M. Sarg, M. M. El-Domiaty, A. A. Ahmed, F. R. Melek and F. Bohlmann, Phytochem., 26, 12, 3356-58 (1987).
10- S. M. H. Ayoub and O. E. Elassam, Fitoterapia, 52, 247 (1981).
11- T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids", Springer Verlag, Heidelberg (1970).
12- L. E. Urbatsch, T. J. Mabry, M. Miyakaza, N. Ohno and A. Yoshioka, Phytochem., 15, 440-41 (1976).
13- T. D. Bacon, L. E. Urbatsch, L. H. Bragg, T. J. Mabry, P. Newman and D. W. Jackson, Phytochem., 17, 1939 (1978).
14- B. Voirin, Phytochem., 22, 2107-45 (1983).
15- J. B. Harborne, T. J. Mabry and H. Mabry, "The Flavonoids", Chapman & Hall, London, 68 (1975).
16- E. Rodriguez, N. J. Carman, G. U. Velde, J. H. McReynolds, T. J. Mabry, T. A. Geisman and M. A. Irwin, Phytochem., 11, 3509 (1972).
17- P. K. Agrawal, S. K. Agrawal and R. P. Pastoga, Phytochem., 19, 893 (1980).
18- Von C. H. Brieskorn and W. Riedel, Planta Medica, 31, 309-10 (1977).