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PHENOLIC CONSTITUENTS FROM RUMEX BUCEPHALOPHORUS GROWING IN LIBYA

Hosny Abd El-Fattah, Sameeh El-Dahmy*, Mahmoud Abdel-Aal, Ahmed F. Halim and Osama B. Abdel-Halim****

Pharmacognosy Department, Faculty of Pharmacy, Al-Fateh University, Libya
** Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt*
*** Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Egypt*

ABSTRACT

The aerial parts of Rumex bucephalophorus yielded 5,7,3',4'-tetrahydr oxyflavan-3-ol together with nine anthraquinones and flavonoids; chrysophanol, emodin, aloe-emodin, isovitexin, iso-orientin, orientin, querecetin, kampferol 3-0-glucoside and luteolin-7-0-glucoside. The structure of these compounds was established on the basis of their spectral characters.

INTRODUCTION

Rumex bucephalophorus is a small herbaceous plant widely growing in Libya, known by the natives as Hammad and is used as laxative like other species of Rumex(1,2). Previous investigation of plants belonging to the genus Rumex revealed the presence of several anthraquinones and flavonoids(3-7). In the present study, we report the first isolation of a flavan-3-ol in this family, in addition to several known anthraquinones and flavonoids of chemical and biological importance as well.

EXPERIMENTAL

General procedures: Mps are uncorrected and determined on Mel-Temp II laboratory devices melting point apparatus; IR spectra were recorded (in KBr) using Pye Unicam Sp 1000 infrared spectrophotometer. UV was recorded on Perkin-Elmer 550 S spectrophotometer, ^{1}H NMR and ^{13}C NMR spectra (PRUKER WM 400 spectrometer) were recorded at 300 and 75.5 MHz, respectively. The EIMS (VG-70, U.K) was measured at 70 ev; FABMS was recorded using glycerol + DMSO-d_6 as liquid matrix; TLC was carried out on Si gel plates (Kiselgel 60, GF 254) using Pet. ether-EtOAc (3:1) and EtOAc-MeOH-H_2O (100 : 15 : 10), also PC. was developed in 15% acetic acid.

Plant material: The flowering aerial parts of Rumex bucephalophorus L. was collected from Tripoli, Libya in March 1992 and kindly identified by Dr. S. Jafri, Faculty of Science, Al-Fateh University. A voucher specimen has been deposited in Pharmacognosy Department, Faculty of Pharmacy, Al-Fateh University, Libya.

Extraction and isolation: The aerial parts (2 Kg) of R. bucephalophorus L. were exhaustively extracted with 75% methanol by percolation (5 x 2 L) at room temperature. The alcoholic extract was concentrated under reduced pressure. The solvent-free extract was diluted with water and successively extracted with light petroleum (b.p. 60-80°), ether and ethyl acetate. The aqueous mother liquor was treated with saturated solution of lead acetate and filtered. The precipitate was washed with water, suspended in methanol and decomposed with H_2S and filtered. The filtrate was evaporated to yield 2.1 g of purified flavonoid extract. This crude flavonoid extract

yielded compounds **9-10** by PC in 15% acetic acid.

The ether extract was chromatographed on silicic acid column (Merck, Type 60, mesh 70-230). Gradient elution was performed by Pet. ether-EtOAc. The separated fractions were concentrated and monitored by TLC plates (Merck, Kiselgel 60, F254, 0.25 mm) in Pet. ether-EtOAc (3:1). Four compounds **1-4** were isolated.

The EtOAc extract was chromatographed on Si gel CC. as described above to afford compounds **5-8.**

Isolation of compound 4 : Column chromatography of the ether extract was eluted with ethyl acetate to give compound **4.** Further purification on repeated Si gel CC afforded white crystals (82 mg) with mp 120-121°C; EIMS : m/z (%): 290 (M^+, 22), 204(30), 152(48), 148(34), 55(13); FABMS : m/z : 291$[M+H]^+$, 313 $[M+Na]^+$; IR: ν_{max} (KBr) : 3450, 1610, 1530, 1475, 1290, 1150, 990, 825 cm^{-1}.

Isolation of the flavonoids: Column chromatography of the EtOAc extract was further purified by PC (15% acetic acid) to give four compounds **5-8** namely: isovitexin (34 mg), as yellow powder (methanol) with mp 246-248°C; isoorientin (32 mg) as yellow powder (methanol) with mp. 235-237°C; orientin (38 mg) dark yellow powder (methanol), mp. 260-262°C kampferol 3-0-glucoside **9** (55 mg) as yellow crystals (methanol), mp. 166-168°C and luteolin 7-0-glucoside **10** (45 mg) as yellow crystals (methanol) with mp. 238-240°C.

Isolation of the anthraquinones: These were isolated from ether extract by elution from the CC. in the following order : chrysophanol **1,** eluted by 5% EtOAc in pet ether as yellow crystals (acetone) (42 mg) with mp. 185-187°C; emodin **2** eluted by 15% EtOAc in pet. ether (35 mg) as dark red prisms (acetone) with mp. 251-253°C; aloe-emodin **3** eluted by 30% EtOAc in pet. ether (25 mg) as faint brown crystals (methanol) with mp. 218-220°C.

Oxidation of flavonoids 5, 6 and 7 using ferric chloride :

About 15 mg of C-flavonoid was subjected to the $FeCl_3$ oxidation(11,15). The sugar moiety was identified by TLC using cellulose pre-coated plates (Merck) using Ethyl acetate : pyridine : n-butanol : acetic acid : water (25:20:20:50:10) solvent system (2 runs)(16) and aniline hydrogen phthalate spray reagent. The chromatograms were visualized by heating at 110° for 5 min. Glucose was detected in each case (R_f 0.46).

RESULTS AND DISCUSSION

The alcoholic extract of the aerial parts of R. bucephalophorus L. afforded after extensive fractionation and chromatography : chrysophanol **1,** emodin **2,** aloe-emodin **3,** isovitexin **5,** iso-orientin **6,** orientin **7,** quercetin 8, kampferol 3-0-glucoside **9** and luteolin 7-0-glucoside **10.** These compounds were identified by their physicochemical properties (mp, ir, uv, ms) and comparison with reported data(7-11).

Compound **4** was isolated from the ether extract showed molecular formula $C_{15}H_{14}O_6$ (EIMS, FABMS). The IR spectrum displayed a strong aromatic band at 1610 cm-1 and a band at 3440 cm-1, indicated the presence of phenolic function.

The ^{1}H-NMR (Table 1) revealed the presence of three coupled aromatic protons resonated at δ 6.7 (1H, d, j = 1.6 Hz), δ 6.6 (1H, d, j = 8.0 Hz) and δ 6.58 (1H, dd, j = 8, 1.6 Hz) corresponding to those of ring B (H-2, H-5 and H-6, respectively). In addition, the pair of doublets at δ 5.87 (j = 2.0 Hz) and δ 5.67 (j = 2.0 Hz), assigned to the meta-coupled protons, H-8 and H-6, respectively (ring A).

The high resolution ^{1}H NMR spectrum of compound **4** was characteristic of a flavan-3-ol nucleus(12). The C-2 pro-

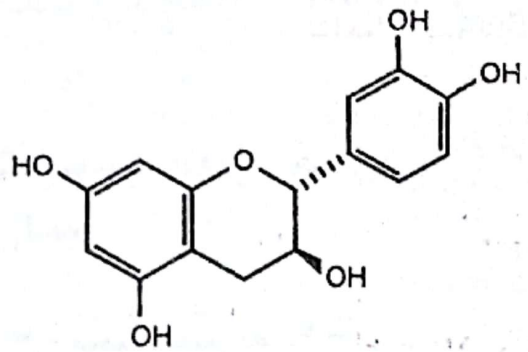
Table (1) : ^{1}H-NMR, ^{13}C-NMR, DEPT and Heterocosy Spectra* of 4.

Position	^{1}H-NMR			^{13}C-NMR		^{1}H-^{13}C cosy
	δ (ppm)	multiplicity	J(HZ)	δ (ppm)	multiplicity (Dept)	
2	4.46	d	7.4	81.0	CH	H-2ß, C-2
3	3.80	m		66.3	CH	H-3α, C-3
4	2.32	dd	8.5, 16	27.8	CH2	H-4a, H-4b, C-4
	2.65	dd	5.3, 16			
5	-	-	-	156.4	C	H-6, C-6
6	5.87	d	2.0	93.8	CH	H-7, H-7
7	-	-	-	156.2	C	H-8, H-8
8	5.67	d	2.0	95.0	CH	-
9	-	-	-	155.3	C	-
10	-	-	-	99.0	C	-
1	-	-	-	130.6	C	H-2', C-2'
2	6.7	d	1.6	114.5	CH	-
3	-	-	-	144.5	C	-
4	-	-	-	144.5	C	H-5', C-5'
5	6.60	d	8.0	115.0	CH	H-6', C-6'
6	6.58	dd	8.0, 1.6	118.4	CH	

* Spectra were recorded in DMSO-d_6 using TMS as internal reference.

ton appeared as a doublet at δ 4.46 (1H, j = 7.4 Hz). On the other hand, the ABX system is diagnostic for the C-3 and C-4 protons. The C-3 proton, the X part, appeared at 3.8 (1H, m). Also, the C-4 protons, the AB part, appeared at δ 2.32, 2.65 (J_{AB} =17, J_{AX} = 8.6, J_{BX} = 5.2 Hz). The low value (j= 8.6 Hz) for the coupling constant J_{AX} was indicative of an axial equatorial coupling. Therefore, the C-3 hydrogen was equatorial and the C-3 OH was axial. This was confirmed by the absence of an absorption band at 795-800 cm^{-1} region of the IR spectrum, characteristic for the cis configuration at C-2 and C-3 in the heterocyclic ring of the epicatechin(13). Therefore, compound **4** was suggested to be catechin type skeleton.

The ^{13}C-NMR spectrum (Table 1) showed the presence of 15 carbons.



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Determination of multiplicity was attained by DEPT and off resonance experiments which revealed the presence of 7 methine, one methylene and 7 non-protonated carbons. All assignments of protons and carbons were confirmed through 2D H-C correlation experiment (Table 1). The carbon assignments are in full agreement with those reported for catechin(14).

From the previously mentioned data (mp, ir, ms, ^{1}H-NMR and ^{13}C-NMR), compound 4 was established to be catechin 5,7,3',4'- tetrahydroxyflavan-3-ol. This isolation represents the first report of this compound in the family.

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المحتويات الفينولية من نبات روميكس بوسيفلوفورز (الحمض) الذى ينمو فى ليبيا

حسنى عبد الفتاح* - سميح الدهمى** - محمود عبد العال* -
أحمد فؤاد حليم+ - أسامة عبد الحليم+

قسم العقاقير - كلية الصيدلة - *جامعة الفاتح - ليبيا - و**جامعة الزقازيق + والمنصورة - مصر

ينمو نبات الحمض بكثرة في ليبيا وهو يستخدم فى الطب الشعبى الليبى كملين. وبدراسة محتوياته الكيميائية تم فصل بعض مركبات الانثراكينون والفلافونيدات الحرة وعلى هيئة جليكوسيدات وقد تم التعرف على هذه المركبات بواسطة الصفات الطبيعية والكيميائية واطياف الأشعة تحت الحمراء والرنين النووى المغناطيسى وطيف الكتلة.