Chemistry of Natural and Microbial Products Department[1] and Chemistry of Tannins and Proteins Department[2], National Research Centre, Dokki, Cairo, Egypt and Pharmacognosy Department[3], Faculty of Pharmacy, Cairo University, Cairo, Egypt

A new flavone diglycoside from *Carthamus tinctorius* seeds

KH. M. AHMED[1], M. S. MARZOUK[2], E. A. M. EL-KHRISY[1], S. ABDEL WAHAB[3] and S. S. EL-DIN[1]

From the aqueous ethanol extract (AE) of *Carthamus tinctorius* seeds, a new acacetin diglycoside has been isolated and identified as acacetin 7-*O*-β-D-apiofuranosyl-(1‴ → 6″ instead of 6′)-*O*-β-D-glucopyranoside together with previously isolated kaempferol 7-*O*-β-D-glucopyranoside, acacetin 7-*O*-α-L-rhamnopyranoside and acacetin. The structures of these metabolites have been established on the basis of chemical, chromatographic and spectral methods.

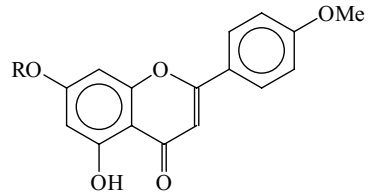
1. Introduction

Carthamus species (Asteraceae) are known in Egyptian folk medicine as analgesics, antiinflammatories and tranquilizers [1]. This genus also has many uses in internal medicine, e.g. for coronary artery disease as it stimulates circulation. It should not be given to pregnant woman as it stimulates the uterus. Externally, it is used for bruising, skin inflammations, wounds and painful or paralyzed joints [2]. Several extracts of *C. tinctorius* L. seeds showed wide safety margins and significant hypoglycemic, analgesic and antiinflammatory activities. Three methoxylated flavonoids as well as the 7-*O*-β-D-glucosides of quercetin, apigenin, oroxylin and quercetin 3-methyl ether were isolated from *C. glaucus* during two previous phytochemical investigations [3, 4]. From *C. oxyacantha* luteolin 7-*O*-β-D-glucoside has been identified [5], while quercetin, luteolin and apigenin [6, 7] have previously been isolated from the flowers and seeds of *C. lanatus*. *C. tinctorius* is also known to be rich in luteolin 7-*O*-β-D-glucoside [8–10] and kaempferol 3-*O*-β-D-rutinoside [11]. Here, we report the isolation, and structural elucidation of a new acacetin diglycoside (**1**) and three known flavonoids (**2**–**4**) from the aqueous ethanol extract of *C. tinctorius* seeds.

2. Investigations, results and discussion

2D-PC Chromatography of the AE extract of the defatted dry powdered seeds revealed the presence of four major flavonoids. The AE extract was chromatographed on a Polyamide column using H_2O and H_2O-EtOH mixtures with decreasing polarity and the fractions obtained were subjected to a combination of repeated PPC and Sephadex LH-20 columns to isolate four flavonoids (**1**–**4**). On the basis of direct chromatographic comparison with respective reference samples, acid hydrolysis and spectral analysis compounds **2**–**4** were identified as kaempferol 7-*O*-β-D-glucoside, acacetin 7-*O*-α-L-rhamnoside and acacetin.

Compound **1** was isolated as a dull yellow amorphous powder and gave chromatographic properties of a flavone glycoside. Its NaOMe UV spectrum showed bathochromic shift with a decrease in the intensity of band I indicating a substituted 4′-OH group. The $AlCl_3$, $AlCl_3$/HCl, NaOAc and NaOAc/H_3BO_3 UV spectra exhibited the characteristic features of a 5-hydroxy-7,4′-di-*O*-substituted flavone [12]. On acid hydrolysis, **1** gave acacetin as an aglycone along with apiose and glucose. Moreover, its CI-MS spectrum showed two significant ionic peaks at m/z 447 and 285 amu, identified as [M-pentose + H]$^+$ and [M-pentosylhexose + H]$^+$, respectively. These results led to the suggestion that in the structure of **1** the glucose moiety is directly attached to the acacetin as an aglycone and the apiosyl moiety is terminal on it, and **1** was accordingly assumed to be acacetin 7-*O*-apiosylglucoside.



1

The ^{1}H NMR spectrum of **1** refered to the presence of a 4′-methoxylated B-ring through a pair of two *ortho* doublets each integrated to two protons at δ ppm 8.0 ($J = 8.5$ Hz) for H-2′/-6′ and a downfield shifted resonance relative to that of apigenin at 7.1 ($J = 8.5$ Hz), which was assigned to H-3′/-5′ together with a singlet integrated for three protons at 4.1 assignable to *O*-Me at C-4′. The *O*-glycosylation at C-7 was deduced from the downfield locations of both H-6 and H-8 resonances as two *meta* doublets at 6.38 ($J = 2.1$ Hz) and 6.8 ($J = 2.1$ Hz), respectively. Additionally, H-3 resonance was detected as a singlet integrated to one proton at 6.8. The glycoside moiety of **1** was suggested to be 7-*O*-β-apiofuranosyl-*O*-β-glucopyranoside because of the two anomeric proton signals, which were investigated as two overlapped doublets at 5.3.

Further confirmation of the structure was obtained from ^{13}C NMR analysis. The spectrum showed two signals at δ ppm 164.8 and 161.8 which were assigned to C-7 and C-4′ indicating glycosylation and methoxylation at these two carbons, respectively. Also, anomeric carbon resonance of the glucose (C-1″) was observed at 100.3, whereas C-1‴ resonance of the apiosyl moiety was detected at 109.6. This typical location of C-1‴ resonance together with that of C-6″ at 67.6, which shifted downfield by ca. 7 ppm in comparison with the corresponding signal of tilianin (60.6) [13] resulted in the conclusion that the apiosyl moiety was terminal attached as (1‴ → 6″) on glucose. This evidence was also supported by the typical location of C-5″ resonance at 64.9 of the free CH_2OH group carbon in

Table 1: ^{13}C NMR data (50 MHz, DMSO-d_6) of 1

C-No. Aglycone	δ ppm	C-No. Sugar	δ ppm
2	163.6	Glucose	
3	104.5		
4	182.9	1″	100.3
5	163.3	2″	72.4
6	99.0	3″	76.9
7	164.8	4″	70.7
8	95.5	5″	76.7
9	157.8	6″	67.6
10	106.2		
1′	123.4	Apiose	
2′	129.4		
3′	115.5	1‴	109.6
4′	161.8	2‴	77.5
5′	115.5	3‴	80.1
6′	129.4	4‴	74.7
OMe	56.4	5‴	64.9

the apiofuranosyl moiety. The remaining carbon resonances of **1** were completely assigned (Table 1) by comparison with the ^{13}C-NMR data of apiine [14] and other structurally related flavonoid mono- and diglycosides [15 to 17]. From the spectral data described above, the structure of **1** was concluded to be acacetin 7-*O*-β-D-apiofuranosyl-(1‴ → 6″)-*O*-β-D-glucopyranoside.

3. Experimental

3.1. Equipment

UV-analyses were run on a Shimazu UV-240 spectrometer and 4 ml quartz cells (1 cm optical pathway). Analytically pure MeOH was used with each of the shift reagents added separately. CI-MS spectrometry was measured on a Finnigan MAT SSQ 7000 spectrometer (Finnigan, Bremen, Germany). NMR analyses were measured on a JEOL EX-270 MHz spectrometer. ^{1}H NMR resonances were recorded relative to TMS scale and ^{13}C relative to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions of spectral width are 4000 for ^{1}H and 17500 Hz for ^{13}C, 32 K data points and a flip angle of 45^0. PC-chromatography was carried out on Whatman No. 1 and 3 MM paper using solvent systems a.15% AcOH, b. n-BuOH-AcOH-H_2O (4 : 1 : 5, top layer) and c. C_6H_6-n.BuOH-H_2O-C_5H_5N (1 : 5 : 3 : 3, top layer).

3.2. Plant material

C. tinctorius L. seeds were collected in May 1996 from the experimental farm, Faculty of Pharmacy, Cairo University and authenticated by Dr. M. El-Gebaly, Chemistry of Natural and Microbial Products Dept., NRC, Cairo, Egypt. A voucher specimen is deposited at the herbarium of the NRC.

3.3. Extraction and isolation

The dry powder of *C. tinctorius* seeds (1 kg) was defatted with $CHCl_3$, then extracted with 70% EtOH to give 59 g dry extract. The concentrated extract was chromatographed on a Polyamide 6S column (Riedel-De Haen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O-EtOH mixtures of decreasing polarity. The major flavonoid fractions obtained were further fractionated using successive PPC and Sephadex LH-20 to give a pure (60 mg) sample of **1**.

3.4. Acacetin 7-O-β-D-apiofuranosyl-(1‴→ 6″)-O-β-D-glucopyranoside (**1**)

R_f-values: [15% AcOH, 0.48 and n-BuOH-AcOH-H_2O (4 : 1 : 5, top layer), 0.44]. UV λ_{max} (MeOH): 270, 324; + NaOMe: 246 sh, 287, 357; + $AlCl_3$: 280, 303, 345, 383; + $AlCl_3$/HCl: 281, 299, 339, 380; + NaOAc: 269, 325; + NaOAc/H_3BO_3: 270, 329 nm. ^{1}H NMR (270 MHz, DMSO-d_6): δ ppm 8.0 (2 H, d, J = 8.5 Hz, H-2′/-6′), 7.1 (2 H, d, J = 8.5 Hz, H-3′/-5′), 6.8 (1 H, d, J = 2.1 Hz, H-8), 6.8 (1 H, s, H-3), 6.38 (1 H, d, J = 2.1 Hz, H−6), 5.3 (2 H, overlapped two doublets, H-1′/-1‴), 4.1 (3 H, s, OMe). ^{13}C NMR: Table 1.

Acid hydrolysis of **1**: A solution of 8 mg in 10 ml 2 N HCl/MeOH-H_2O was heated under reflux at 100 °C for 2 h. After cooling, the reaction mixture was exhaustively extracted with EtOAc. The aglycone was identified from the EtOAc extract as acacetin (CoPC), while the aqueous hydrolysate was neutralized, concentrated and then examined by CoPC to confirm the presence of apiose and glucose.

References

1 Taeckholm, V.: Student's Flora of Egypt, p. 544, Cairo University, Egypt, Cooperative printing Co., Beirut 1974
2 Brown, D.: Encyclopaedia of Herbs and their uses, Herb Society of America, 1st American ed. p. 544, Dorling Kindersley, Inc. New York, London 1995
3 Kafagy, S. M.; Sabri, N. N.; Abd El-Salam, N.; Seif El-Din, A.: Acta Pharm. Jugosl. **29**, 161 (1979)
4 Seif El-Din, A.: Acta Pharm. Jugosl. **37**, 97 (1987); C.A. **107**, 55791s (1987)
5 Kapoor, S. K.; Khan, H.; Zaman, A.: Indian J. Appl. Chem. **33**, 262 (1970)
6 Reynaud, J.; Becchim, M.; Prum, N.: Plant Med. Phytother. **10**, 170 (1976)
7 El-Kayaat, S. A.; Lahloup, M. F.; Amer, M. M.; Haraz, F.: Mansoura J. Pharm. Sci. **9**, 234 (1993)
8 Tashichiro, N.; Naokata, M.: Yakugaku Zasshi. **80**, 1473 (1960)
9 Ismailov, N. M.; Kuliev, A. A.: Dokl. Akad. Naukaz. SSR. **41**, 61 (1985)
10 Homma, T.; Nasure, A.; Matsuchisa, Y.: J. Adv. Sci. **2**, 77 (1990)
11 Murti, V. V. S.; Raman, P. V.; Seshadri, T. R.; Thakur, R. S.: J. Sci. Ind. Res. **21B**, 80 (1962)
12 Mabry, T. J.; Markham, K. R.; Thomas, M. B.: The Systematic Identification of Flavonoids, Springer, Berlin 1970
13 Agrawal, P. K.; Thakur, R. S.; Bansal, M. C.; in: Agrawal, P. K. (ed.): ^{13}C NMR of Flavonoids, chapter **6**, p 320, Elsevier, New York 1989
14 Forgacs, P.; Desconclois, J. F.; Pousset, J. L.; Rabaron, A.: Tetrahedron Letters **48**, 4783 (1978)
15 Markham, K. R., Ternai, B.; Stanley, R.; Geiger, N.: Tetrahedron **34**, 1389 (1978)
16 Nakano, K.; Takatani, M.; Tomimatsu, T.; Nohara, T.: Phytochemistry **22**, 2831 (1983)
17 Cui, B.; Nakamura, M.; Kinjo, J.; Nohara, T.: Chem. Pharm. Bull. **41**, 178 (1993)

Received May 17, 1999
Accepted December 12, 1999

Dr. M. S. Marzouk
Chemistry of Tannins and Proteins Dept.
National Research Centre
Tahrir Str.
Dokki, Cairo
Egypt
msmarzouk@yahoo.co.uk