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FLAVONOIDS OF THE FLOWERS OF *SILYBUM MARIANUM*

AHMED A. AHMED, TOM J. MABRY* and STEPHEN A. MATLIN†

Department of Chemistry, Faculty of Science, El-Minia University, El-Minia, Egypt, *The Department of Botany, The University of Texas at Austin, Austin, TX 78713 U.S.A.; †Department of Chemistry, The City University, Northampton Square, London EC1V OHB, U.K.

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Key Word Index—*Silybum marianum*, Compositae; Cardueae; flowers, apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl) galacturonide, kaempferol 3-*O*-α-rhamnoside-7-*O*-β-galacturonide, apigenin 7-*O*-β-glucuronide 6″-ethyl ester

Abstract—Seven flavonoids are reported from an aqueous methanol extract of the flowers of *Silybum marianum*: apigenin 7-*O*-β-(2″-*O*-α-rhamnosyl)galacturonide, kaempferol 3-*O*-α-rhamnoside-7-*O*-β-galacturonide, apigenin 7-*O*-β-glucuronide 6″-ethyl ester, apigenin 7-*O*-β-glucoside, apigenin 7-*O*-β-galactoside, kaempferol-3-*O*-α-rhamnoside and kaempferol. The structures were established by routine methods as well as by FABMS and ^{13}C NMR spectral measurements.

INTRODUCTION

Silybum Adans. (Compositae, Tribe Cardueae) is a genus with only two species, both of which are common in the Mediterranean regions [1]. *Silybum marianum* (L.) Gaertn. (syn. *Carduus marianus* L.) is used medicinally [2] in many countries. Therefore, *S. marianum* has been subjected to many chemical investigations [3–6]. We have now studied the flavonoids of the flowers. We here describe seven, including three new glycosides.

RESULTS AND DISCUSSIONS

Chromatographic separation of the material from the aqueous methanol extract of the flowers of *S. marianum* afforded three new flavonol glycosides (**1–3**) and four known flavonoids. The flavonoids were isolated by CC and prep. PC. The UV spectra were recorded with diagnostic reagents [7].

Compound **1** had the spectral and colour properties of an apigenin 7-glycoside and on acid hydrolysis yielded apigenin, rhamnose and galacturonic acid, all of which were identified by co-chromatography with authentic samples. In addition, the negative ion FABMS showed the molecular ion $[M-H]^-$ at *m/z* 591, further supporting an apigenin nucleus with galacturonic acid and rhamnosyl moieties. The fragmentation pattern showed a peak at *m/z* 445 in accord with the loss of a terminal rhamnose group, while a peak appeared at *m/z* 269 indicative of the loss of the disaccharide moiety. ^{1}H NMR data (as the trimethylsilyl ether in $CDCl_3$) showed the apigenin skeleton: two-proton doublet for H-2′, 6′ at δ7.8 ($J=9$ Hz) coupled to another doublet at δ6.9 ($J=9$ Hz) for H-3′, 5′. A narrow *meta*-coupled doublet at δ6.65 ($J=2.5$ Hz) and 6.5 ($J=2.5$ Hz) for H-8 and H-6, respectively. Additionally, H-3 appeared as a singlet at δ6.6. The signals at δ5.1 ($J=2.5$ Hz, one proton) and δ1.1 ($J=6$ Hz, three protons) could be assigned to the anomeric proton and the methyl group, respectively, of the rhamnosyl moiety. A signal at δ5.05 with a coupling constant of 7.5 Hz was in accord with an anomeric proton of a β-galacturonic acid moiety. The appearance of the signals for the anomeric proton and the methyl group of the rhamnosyl moiety at δ5.1 and δ1.1, respectively, supported a 1→2 linkage rather than a 1→6 linkage [8]. Moreover, the sugar sequence was confirmed by ^{13}C NMR data, which were in agreement with those reported for (2″-*O*-rhamnosyl)-galacturonide [9] (Table 1). The two anomeric carbon atoms of the galacturonic acid and rhamnosyl groups appeared at δ99.5 and 100.8, respectively. Thus, **1** was identified as apigenin 7-*O*-β-D-(2″-*O*-α-L-rhamnosyl)-galacturonide, a new natural product.

Acid hydrolysis of **2** with 1 N TFA afforded the aglycone kaempferol as well as the sugars rhamnose and galacturonic acid. Spectral and colour reactions of **2** indicated 3,7-disubstitution. Additionally, alkaline hydrogen peroxide oxidation produced rhamnose, while enzymatic treatment with pectinase gave a product which was identified by UV and ^{1}H NMR (as the trimethylsilyl ether in $CDCl_3$) as kaempferol 3-*O*-α-L-rhamnoside. These results indicated the linkage of rhamnose to the 3-*O*-position and galacturonic acid at the 7-*O*-position. This conclusion was confirmed by ^{1}H NMR (as the trimethylsilyl ether in $CDCl_3$) which demonstrated two doublets at δ5.2 ($J=2.5$ Hz) and δ5.05 ($J=9$ Hz) for the anomeric protons of the rhamnosyl and galacturonic acid moieties, respectively. The identity of the galacturonic acid moiety was confirmed by ^{13}C NMR (Table 1). Furthermore, negative ion FABMS yielded a molecular peak at *m/z* 608, indicative of a kaempferol nucleus with rhamnosyl and galacturonic moieties. Additional peaks appeared at *m/z* 460 and *m/z* 431 for $[M-\text{rhamnose-H}]^-$ and $[M-\text{galacturonic}]^-$, respectively. Therefore, **2** is kaempferol 3-*O*-α-L-rhamnoside-7-*O*-β-D-galacturonide, a new flavonol glycoside.

Compound **3** exhibited chromatographic properties and UV spectral data similar to those of a flavone 7-*O*-glycoside. Prolonged acid hydrolysis produced apigenin and glucuronic acid, which were identified by co-chro-

Table 1 ^{13}C NMR data of the flavonoids **1–4**

C	1	2	3	4
2	164 49	156 36	164 30	164 33
3	102 68	134 10	103 12	103 10
4	182.05	175.07	181 93	181 99
5	162 04	162 84	161 08	161 14
6	97 59	99 34	99 18	99.30
7	162 63	162 84	162 40	162 62
8	94.44	94.00	94 70	94 72
9	157 12	156 36	156 94	156 97
10	105 59	101 76	105 47	105.43
1′	120 55	120 08	120 91	120 93
2′	128 48	130 36	128 58	128 60
3′	116 19	115 84	115 99	116.03
4′	161 21	160 28	161 45	161 43
5′	116 19	115 84	115 99	116 03
6′	128 48	130 36	128.58	128 60
1″	99 52	99 69	99 32	99 44
2″	77 37	73.95	71 24	71 46
3″	74 20	72 99	72 72	72.81
4″	68 54	69 76	75 40	75 84
5″	76 54	76.50	75 22	75 10
6″	172 10	172 29	168 67	170 97
1‴	100 78	99 69		
2‴	70 72	70 57*		
3‴	70 72*	70 08*		
4‴	72 10	71 89		
5‴	70 59*	70 37*		
6‴	18 25	17 52		
O–CH_2–			60.77	
–Me			13 95	

*Interchangeable within the column

matography with authentic samples. ^{1}H NMR of **3** exhibited signals for a flavonoid skeleton of the apigenin type, while the doublet at δ5.1 (J=8 Hz) could be assigned to the anomeric proton of the sugar moiety with a β-configuration. Additional signals appeared as a quartet at δ4.2 and a triplet at δ1 3, typical for ethyl ester substitution

In the ^{13}C NMR spectrum, ethyl esterification was indicated by the two signals at δ13 95 and δ60.76. Mild acid hydrolysis of **3** yielded **4**, which was identified by UV, ^{1}H NMR and ^{13}C NMR as apigenin 7-*O*-β-D-glucuronide Comparison of ^{13}C NMR of **3** and **4** showed a small upfield shift (Δ 2.3 ppm) of C″-6, indicating esterification of the carboxylic group with an ethyl group [10].

EXPERIMENTAL

Plant material Silybum marianum (L) Gaertn was collected in Egypt near the El-Minia University campus in March 1987. A voucher specimen is deposited in the Department of Botany, El-Minia University, Egypt

General CC employed Polyclar AT, microcrystalline cellulose (Avicel) and Sephadex LH-20 The solvent system for PC and TLC was TBA (*t*-BuOH–HOAc–H_2O), 3·1 1, 15% and 30% HOAc Visualization of the flavonoids on PC and TLC was realized either by UV light + NH_3 or by spraying with NA in MeOH All UV data were recorded using standard procedures [7] ^{1}H NMR spectra of the trimethylsilyl ethers of all flavonoids were recorded in $CDCl_3$ at 200 MHz and are reported as δ-values (ppm) relative to TMS as an internal standard, while ^{13}C NMR were recorded in DMSO-d_6 at 125 MHz

Isolation and characterization of flavonoids The fresh flowers of *S. marianum* were extracted with 80 and 50% aq. MeOH The extract, combined and concd, was chromatographed over Polyclar AT (GAF Corp), eluted first with H_2O and then with increasing amounts of MeOH

All compounds were purified over Sephadex LH-20 prior to analysis by UV, ^{1}H and ^{13}C NMR Acid hydrolysis of the glycosides (1 N TFA, 7 hr) yielded, depending on the compound, rhamnose, galacturonic acid, glucuronic acid, galactose, glucose, apigenin or kaempferol, all of which were cochromatographed with authentic samples

Apigenin 7-O-β-D-(2″-O-α-L-*rhamnosyl*)*galacturonide* (**1**) R_f values, TBA, 0.22, 15% HOAc, 0 62 UV λ_{max}^{MeOH} nm 268, 334, NaOMe, 264, 290sh, 386; $AlCl_3$, 275, 295, 345, 389, $AlCl_3$–HCl, 274, 295, 345, 389, NaOAc, 266, 340, NaOAc–H_3BO_3, 265, 335 ^{1}H NMR (as Me_3Si ether): δ7 8 (*d*, J=9 Hz, H-2′, 6′), 6 9 (*d*, J =9 Hz, H-3′, 5′), 6 65 (*d*, J=2 5 Hz, H-8), 6 6 (*s*, H-3), 6.5 (*d*, J =2 5 Hz, H-6), 5 1 (*d*, J=2 5 Hz, H-1, rhamnosyl), 5 05 (*d*, J =8 Hz, H-1, galacturonic acid), 4 0–3 5 (*m*, sugar protons), and 1.1 (*d*, J=6 Hz, –Me, rhamnose)

Kaempferol 3-O-α-L-*rhamnoside* 7-O-β-D-galacturonide (**2**). R_f values, TBA, 0 20, 15% HOAc, 0 72 UV λ_{max}^{MeOH} 267, 350. NaOMe, 278, 305sh, 350sh, 405, $AlCl_3$, 274, 350, 394, $AlCl_3$–HCl, 274, 350, 394, NaOAc, 267, 357, 410sh, NaOAc–H_3BO_3, 267, 352 ^{1}H NMR (as Me_3Si ether) δ7 75 (*d*, J =9 Hz, H-2′, 6′), 6 9 (*d*, J=9 Hz, H-3′, 5′), 6 5 (*d*, J=2 5 Hz, H-8), 6 2 (*d*, J=2 5 Hz, H-6), 5.2 (*d*, J=2 5 Hz, H-1, rhamnosyl), 5 1 (*d*, J=8 Hz, H-1, galacturonic acid), 4 0–3 5 (*m*, sugar protons) and 0 85 (*d*, J=6 Hz, –Me, rhamnose)

Apigenin 7-O-β-D-*glucuronide* 6″-*ethyl ester* (**3**) R_f values, TBA, 0 75, 15% HOAc, 0 18 UV λ_{max}^{MeOH} 266, 333, NaOMe, 250, 265, 295sh, 385, $AlCl_3$, 275, 295, 345, 380. $AlCl_3$–HCl, 275, 295, 346, 382, NaOAc, 268, 335sh, 385, NaOAc–H_3BO_3, 268, 338 ^{1}H NMR (as Me_3Si ether) δ7 8 (*d*, J=9 Hz, H-2′, 6′), 6 95 (*d*, J =9 Hz, H-3′, 5′), 6 6 (*s*, H-3), 6 55 (*d*, J=2 5 Hz, H-8), 6 4 (*d*, J =2.5 Hz, H-6), 5.1 (*d*, J=8 Hz, H-1, glucuronic acid), 4 2 (*q*, O–CH_2, ethyl), 4 0–3.5 (*m*, sugar protons) and 1 3 (*t*, –Me, ethyl)

Apigenin 7-O-β-D-*glucuronide* (**4**) R_f values, TBA, 0 60, 15% HOAc, 0.18 ^{1}H NMR (as Me_3Si ether) δ7 8 (*d*, J=9 Hz, H-2′, 6′), 6 95 (*d*, J=9 Hz, H-3′, 5′), 6 6 (*s*, H-3), 6 6 (*d*, J=2 5 Hz, H-8), 6 4 (*d*, J=2 5 Hz, H-6), 5 1 (*d*, J=8 Hz, H-1, glucuronic acid) and 4 0–3 5 (*m*, sugar protons) The UV data for **4** were similar to those for compound **3**

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