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FLAVONOIDS OF THE FLOWERS OF *TAMARIX NILOTICA*

M A M NAWWAR, A M A SOULEMAN, J BUDDRUS* and M LINSCHEID*

National Research Centre, Dokki, Cairo, Egypt, *Institut fur Spektrochemie und angewandete Spektroskopie, Bunsen-Kirchhoff Str 11, D-4600 Dortmund, W Germany

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Key Word Index—*Tamarix nilotica*, Tamaricaceae, flowers, kaempferol 3-*O*-β-D-glucuronide 6″-ethyl ester, quercetin 3-*O*-β-D-glucuronide 6″-methyl ester, quercetin 3-*O*-β-D-glucuronide 6″-ethyl ester, kaempferol 3-*O*-sulphate-7,4′-dimethyl ether

Abstract—The ethyl ester of kaempferol 3-*O*-β-D-glucuronide, the methyl and ethyl esters of quercetin 3-*O*-β-D-glucuronide have been isolated from an aqueous acetone extract of the flowers of *Tamarix nilotica* In addition kaempferol 3-*O*-sulphate-7,4′-dimethyl ether and the free aglycones were isolated The structures were established by routine methods, by FAB-MS and by ^{13}C NMR spectral measurements

INTRODUCTION

In previous reports, flavonoid and phenolic constituents have been reported from the leaves, roots and flowers of *Tamarix nilotica* [1–3] We have now studied the flavonoids of these flowers, and describe here the identification of three new natural products

RESULTS

Preliminary screening of the aqueous acetone extract of the flowers of *T nilotica* by TDPC indicated the presence of a mixture of phenolic components (ferric chloride spray reagent) from which compounds **1–4** were isolated and purified by CC on polyamide and PPC Compounds **1a**, **2a**, kaempferol, quercetin and kaempferol 7,4′-dimethyl-ether were also isolated and purified in the same way

Compound **1** was isolated as an amorphous yellow powder which exhibited chromatographic properties and UV spectral data (Table 1) similar to those of flavonol 3-*O*-glycosides [4] and with a MW of 490 as shown by FAB-MS (MH$^+$ 491) On acid hydrolysis it yielded kaempferol and glucuronic acid Compound **1** was partially hydrolysed by dilute acid to yield an intermediate **1a** for which the chromatographic and UV analysis (Table 1) showed it to be kaempferol 3-*O*-β-D-glucuronide The structure of **1a** was confirmed by ^{1}H (see Experimental) and by ^{13}C NMR (Table 2) The stability of **1** to enzymatic hydrolysis by β-glucuronidase, the absence of substitution other than that at position 3 (UV data) and the small difference in MW between **1** and **1a** suggested the presence of an ethyl substituent on the glucuronic acid moiety The ^{1}H NMR spectrum of **1** revealed an ethyl group and its structure was confirmed as kaempferol 3-*O*-β-D-glucuronic acid ethyl ester by ^{13}C NMR [5, 6] In the spectrum of **1**, ethyl esterification follows from the two signals at δ14 01 and 60 84 Comparison of the spectra of **1** and **1a** showed a small upfield shift (Δδ 1 2 ppm) of the esterified carboxylic carbon

Compound **2** was separated as yellow amorphous powder It showed all the properties of a flavonol 3-*O*-glycoside had a MW of 492 and was not hydrolysed by β-

Table 1 Chromatographic and UV data of the flavonoids

	Chromatographic properties R_f s (× 100)			UV spectral data				
					Δλ (nm)			
	H_2O	HOAc	BAW	λ_{max} (nm) in MeOH	NaOAc*	NaOAc–H_3BO_3†	NaOMe†	$AlCl_3$†
1	74	48	45	267, 350	7	13	61	37
1a	72	45	42	366, 350	6	13	65	39
2	70	46	37	256, 269‡, 357	8	12	52	41
3	72	46	43	256, 270‡, 357	8	12	50	41
2a	67	41	38	255, 268‡, 357	6	12	55	43
4	54	60	65	268, 340	0	0	37	55

*Band II
†Band II
‡Inflection

Table 2 ^{13}C NMR data of the flavonoids

Carbon number	Kaempferol	1a	1	Quercetin	2a	2	Kaempferol 7,4'-dimethyl ether	4
2	146 8	156 4	156 6	146 9	156 4	156 3	146 7	156 0
3	135 7	133 0	133 4	135 5	134 0	133 2	136 3	132 8
4	175 9	177 2	177 4	175 8	177 6	177 2	176 1	177 9
5	160 7	161 2	161 2	160 7	160 8	161 3	160 7	161 2
6	98 2	98 8	99 0	98 2	98 0	98 8	97 6	97 6
7	163 9	164 3	164 5	163 9	164 7	164 3	165 0	165 0
8	93 5	93 8	94 0	93 3	93 7	93 6	92 2	92 2
9	156 2	156 4	157 0	156 2	157 4	156 3	156 3	156 3
10	103 0	104 0	104 2	103 1	103 6	103 9	104 1	105 2
1'	121 7	120 6	120 8	122 1	120 4	120 6	123 2	122 7
2'	129 5	131 0	131 1	115 3	115 4	115 2	129 5	131 0
3'	115 5	115 2	115 3	145 0	144 8	144 9	114 2	113 6
4'	159 2	160 1	160 4	147 6	148 5	148 6	160 2	161 0
5'	115 5	115 3	115 3	115 6	114 4	116 2	114 2	113 6
6'	129 5	131 0	131 1	120 0	121 0	120 9	129 5	131 0
1"		102 1	101 9		102 8	101 4		
2"		71 4	71 6		71 6	71 4		
3"		74 0	74 2		74 0	73 8		
4"		75 7	75 9		76 49	75 6		
5"		76 0	75 9		76 49	75 6		
6"		169 8	168 6		169 7	168 69		
Ac						51 9		
EtCO			14 0					
EtCO			60 8					
MeO							55 9 and 55 3	56 0 and 55 3

glucuronidase Chromatographic, UV data (Table 1) and ^{1}H NMR analysis (see Experimental) of **2** and its complete and partial acid hydrolysis products showed it to be quercetin 3-*O*-*β*-D-glucuronic acid methyl ester ^{13}C NMR analysis of **2** and its partial hydrolysis product **2a** (Table 2) confirmed its structure Comparison of the C-2 and C-3 signal positions in the ^{13}C NMR spectra of **2**, **2a** and quercetin proved substitution at C-3 of the quercetin moiety In the spectrum of **2**, methyl esterification follows from the signal at δ 51 9 and from the small upfield shift ($\Delta\delta$ 1 ppm) of the esterified carboxyl carbon

Compound **1a** was eluted together with **2** from the column It was isolated as an amorphous yellow powder and identified to be kaempferol 3-*O*-*β*-D-glucuronide by chromatographic and UV data (Table 1), acid and *β*-glucuronidase hydrolysis and by ^{1}H (see Experimental) and ^{13}C NMR (Table 2)

Compound **3**, a minor constituent, was separated as yellow amorphous powder with chromatographic and UV data (Table 1) similar to those of quercetin 3-*O*-*β*-D-glycosides [4] It had a MW of 506 (FAB-MS, MH$^+$ 507) and yielded quercetin and glucuronic acid on acid hydrolysis On controlled acid hydrolysis it yielded quercetin 3-*O*-*β*-D-glucuronide These data suggested it was quercetin 3-*O*-*β*-D-glucuronic acid ethyl ester The ^{1}H NMR spectrum of **3** proved the presence of an ethyl group esterifying the glucuronic acid moiety, thus confirming this structure

Compound **2a** was separated as an amorphous yellow powder which was identified as quercetin 3-*O*-*β*-D-glucuronide by chromatographic and UV data (Table 1), acid and *β*-glucuronidase hydrolysis, and by ^{1}H (see Experimental) and ^{13}C NMR Compound **4** was isolated as white amorphous powder which was found to be identical (CoPC, UV, mp, mmp) with kaempferol 7,4'-dimethyl ether 3-sulphate This compound occurs in the leaves of the same plant [7] Its composition was established by accurate mass measurement in negative FAB-MS of the signal of the anion at *m/z* 393 to be $C_{17}H_{13}O_9S$ (see Experimental) ^{1}H NMR spectrum of **4** was similar to that of its flavonol moiety **4a** (see Experimental) ^{13}C NMR of both **4** and **4a** provided a confirmation of the deduced structure as follows The chemical shifts of the C-atoms in **4** are similar to those of the corresponding C-atoms in **4a** (Table 2), but a distinction can be made since the signals of the C-3 and C-2 of **4** are different from those of the same two carbons in **4a** This change in chemical shift values is obviously due to substitution with the sulphate residue at C-3 In the ^{13}C NMR spectrum of **4**, the absence of other ^{13}C signals, apart from those of the flavonol moiety, supported the presence of an inorganic substituent

EXPERIMENTAL

^{1}H chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts relative to DMSO-d_6 and converted into the TMS scale by adding 39 5 Typical conditions spectral width 5000 Hz 8K data points and a flip angle of 45° For FAB-mass spectrometry a MM 7070E instrument (VG analytical) has been used Accurate masses in FAB-MS were determined with phosphoric acid added as an internal standard to the glycerol matrix (1 10) The resolution was set to M/ΔM = 2000 PC was

carried out on Whatman paper 1 or 3 MM using (1) H_2O, (2) HOAc (HOAc–H_2O, 3 17), and (3) BAW (*n*-BuOH–HOAc–H_2O, 4 1 5, top layer)

Plant material Flowers of *T nilotica* were collected from El-Fayyoum desert in Egypt during Jan 1983 and classified by Dr L Boulos, National Research Centre, Cairo Vouchers are deposited at the N R C

Isolation and identification Samples of the fresh flowers and of flowers dried in the shade in an air draft were individually extracted with H_2O or Me_2CO–H_2O (1 3) The dried extracts were subjected to TDPC and CoPC and gave identical flavonoid patterns The dried extract of the dried ground flowers was applied to a polyamide 6 S column and eluted by H_2O followed by H_2O–MeOH mixtures of decreasing polarities to yield three major fractions

Kaempferol 3-O-β-D-glucuronide 6″-ethyl ester (**1**) Isolated from the 20% aq MeOH column fraction, mp 208°, R_fs and UV spectra Table 1 Acid hydrolysis (1 5 N aq HCl, 100°, 45 min) of **1** gave kaempferol (CoPC and ^{13}C NMR data, Table 2) and glucuronic acid (CoPC), while controlled acid hydrolysis (0 5 N HCl, 100°, 3 min) yielded kaempferol 3-*O*-β-D-glucuronide **1a** (R_fs and UV, Table 1) ^{1}H NMR of **1a** aglycone moiety δ 6 2 (*d*, *J* = 2 5 Hz, 6-H), 6 4 (*d*, *J* = 2 5 Hz, 8-H), 6 84 (*d*, *J* = 8 Hz, 3′-H and 5′-H), 8 (*d*, *J* = 8 Hz, 2′-H and 6′-H), sugar moiety δ 5 48 (*d*, *J* = 9 Hz, 1-H β-glucuronide), 3 2–3 8 (*m*, sugar protons overlapping with OH protons) ^{13}C NMR of **1a** Table 2 ^{1}H NMR of **1** aglycone moiety δ6 2 (*d*, *J* = 2 5 Hz, 6-H), 6 4 (*d*, *J* = 2 5 Hz, 8-H), 6 88 (*d*, *J* = 8 Hz, 3′-H and 5′-H), 8 (*d*, *J* = 8 Hz, 2′-H and 6′-H), sugar moiety δ 5 4 (*d*, *J* = 9 Hz, 1-H β-glucuronide), 3 68 (*d*, *J* = 7 Hz, 5″-H), 3 2–3 7 (*m*, 2″-H, 3″-H and 4″-H overlapping with OH protons), ethyl moiety δ 4 02 (*q*, *J* = 8 Hz, CH_2O protons), 1 08 (*t*, *J* = 8 Hz, Me) ^{13}C NMR of **1** Table 2

Quercetin 3-O-β-D-glucuronide 6″-methyl ester (**2**) Isolated from the 60% aq MeOH column fraction, mp 214°, R_fs and UV, Table 1 Acid hydrolysis gave quercetin (CoPC and ^{13}C NMR data Table 2) and glucuronic acid (CoPC) Controlled acid hydrolysis yielded quercetin 3-*O*-β-D-glucuronide **2a** ^{1}H NMR of **2a** aglycone moiety δ 7 6 (*d*, *d*, J_{meta} = 1 5 Hz, J_{ortho} = 7 Hz, 6′-H), 7 55 (*br s*, Δν 1/2 = 1 5 Hz, 2′-H), 6 86 (*d*, *J* = 7 Hz, 5′-H), sugar moiety δ 5 5 (*d*, *J* = 9 Hz, 1-H β-glucuronide), 3 2–3 9 (*m*, sugar protons overlapped with OH protons) ^{13}C NMR of **2a** Table 2 ^{1}H NMR of **2** aglycone moiety δ 7 6 (*d*, *d*, J_{meta} = 1 5 Hz, J_{ortho} = 7 Hz, 6′-H), 7 5 (*br s*, Δν 1/2 = 1 5 Hz, 2′-H), 6 8 (*d*, *J* = 7 Hz, 5′-H), sugar moiety δ 5 4 (*d*, *J* = 9 Hz, 1-H β-glucuronide), 3 9 (*d*, *J* = 7 Hz, 5″-H), 3 2–3 7 (*m*, 2″-H, 3″-H and 4″-H overlapped with OH protons), methyl moiety δ 3 3 (*s*, Ac)

Kaempferol 3-O-D-glucuronide (**1a**) Isolated from the 60% column fraction as yellow amorphous powder was identified as above

Quercetin 3-O-β-D-glucuronide 6″-ethyl ester (**3**) Represents the minor flavonoid of the 60% column fraction It was isolated pure by repeated prep PC, R_fs and UV, Table 1 Acid hydrolysis gave quercetin (CoPC) and glucuronic acid (CoPC) Controlled acid hydrolysis yielded quercetin 3-*O*-β-D-glucuronide (CoPC and UV spectral data) ^{1}H NMR of **3** aglycone moiety δ 7 64 (*d*, *d*, J_{meta} = 1 5 Hz, J_{ortho} = 7 Hz, 6′-H), 7 55 (*br s*, Δν 1/2 1 5 Hz, 2′-H), 6 84 (*d*, *J* = 7 Hz, 5′-H), sugar moiety δ 5 5 (*d*, *J* = 9 Hz, 1-H β-glucuronide), 3 3–3 95 (*m*, sugar protons overlapped with OH protons), Ethyl ester moiety δ 4 08 (*q*, *J* = 8 Hz, CH_2O protons), 1 12 (*t*, *J* = 8 Hz, Me)

Quercetin 3-O-β-D-glucupyranuronide **2a**. Isolated from the 70% column fraction as yellowish brown amorphous powder of mp 180° It was identified as described above

Kaempferol 7,4′-dimethyl ether 3-sulphate (**4**) Isolated from the 70% column fraction, mp 204° (decomp), R_fs and UV Table 1 Accurate masses in FAB-MS seven scans were averaged and the signals at *m/z* 392, 393 and 394 gave an elemental composition of $C_{17}H_{13}O_9S$ for the anion of the sulphate and provided additional information for establishing its structure 393 02798 (calc), 393 02118 (obs), +6 8 $C_{17}H_{13}O_9{}^{32}S$, 394 03136 (calc), 394 03366 (obs), −2 3 $C_1C_{16}H_{13}O_9{}^{32}S$, 395 02385 (calc), 395 02235 (obs), +1 5 $C_{17}H_{13}O_9{}^{34}S$ mmU The aglycone **4a** was obtained by acid hydrolysis of **4** [7] ^{1}H NMR of **4a** δ 8 16 (*d*, *J* = 8 Hz, 2′-H and 6′-H), 7 1 (*d*, *J* = 8 Hz, 3′-H and 5′-H), 6 74 (*d*, *J* = 1 5 Hz, 8-H), 6 34 (*d*, *J* = 1 5 Hz, 6-H), 3 84 and 3 85 (two *s*, 7 and 4′-OMe) ^{13}C NMR of **4a** Table 2 ^{1}H NMR of **4** δ 8 2 (*d*, *J* = 7 Hz, 2′-H and 6′-H), 7 04 (*d*, *J* = 7 Hz, 3′-H and 5′-H), 6 72 (*d*, *J* = 1 5 Hz, 8-H), 6 34 (*d*, *J* = 1 5 Hz, 6-H), 3 84 (*br s*, 7 and 4′-OMe) ^{13}C NMR of **4** Table 2

Kaempferol, quercetin and kaempferol 7,4′-dimethyl ether were eluted together from the column using MeOH Prep PC was applied for the isolation of each of them Identification was achieved by CoPC, UV spectral data and ^{13}C NMR analysis (Table 2)

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