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FLAVONOID GLYCOSIDES OF *TRIBULUS PENTANDRUS* AND *T. TERRESTRIS*

NABIEL A. M. SALEH, AHMED A. AHMED and MOHAMED F. ABDALLA

National Research Centre, El-Dokki, Cairo, Egypt

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Key Word Index—*Tribulus pentandrus*; *T. terrestris*; Tribulaceae; flavonol glycosides; chemosystematics.

Abstract—Twenty-five flavonoid glycosides were detected in *Tribulus pentandrus* and *T. terrestris*. The glycosides belong to the common flavonols, kaempferol, quercetin and isorhamnetin, with the 3-gentiobiosides as the major glycosides. Traces of a flavone (tricin) glycoside was also present in *T. pentandrus*. The separation of Tribulaceae as a distinct family from Zygophyllaceae is discussed.

INTRODUCTION

Tribulus L. (Tribulaceae Hadidi) is composed of about 25 species [1]. The separation of Tribulaceae from Zygophyllaceae as a distinct family was based on the characters of fruit, seeds and leaves [1, 2]. *Tribulus* species are distributed in the arid and semi-arid zones of the Middle East, Africa, Asia Minor and Australia [1, 3].

The chemistry of members of the Zygophyllaceae R.Br. (s.l.) has been studied by several authors. *Larrea* species have been extensively studied, both in the Old World [4–6] and the New World [7–10]. Furthermore, the flavonoid glycosides of some *Zygophyllum*, *Fagonia*, *Seetzenia*, *Tribulus*, *Nitraria* and *Balanites* species have been identified [11]. The flavonoid patterns in the *Larrea* species studied show a large number of methylated flavonoids [7–10]. On the other hand, members of the Old World Zygophyllaceae seem to have simpler flavonoid aglycones [11]. Furthermore, glycosylation patterns appear to play an important role in the chemosystematics of the Zygophyllaceae [11]. Here we report the detection of 25 flavonoid glycosides in the leaves of *Tribulus pentandrus* and *T. terrestris*.

RESULTS

From the leaves of *Tribulus pentandrus* and *T. terrestris*, 25 flavonoid glycosides were isolated and characterized. The glycosides were found to be based on kaempferol, quercetin, isorhamnetin and tricin. The glycosidic patterns were confirmed through both chemical and physical methods. The distribution of the glycosides in both plants is recorded in Table 1, while results of the chemical and physical analysis are recorded in Tables 1–3.

Four glycosides are reported here for the first time. The 3-gentiobioside-7-glucosides of kaempferol and isorhamnetin have not been previously reported. Quercetin-3-gentiobioside-7-glucoside was first isolated from *Tribulus* species in 1977 [11]. All three glycosides gave the corresponding 3-gentiobiosides on enzymic hydrolysis with β-glucosidase, and the corresponding 7-glucosides on mild acid hydrolysis. R_f values are in agreement with those reported for triglycosides [12] as well as the corresponding isomeric 3-glucoside-7-gentiobiosides of kaempferol, quercetin and isorhamnetin [13].

The third new glycoside was identified as quercetin-3-rhamnogentiobioside. It gave the corresponding 3-gentiobioside on mild acid hydrolysis, and a trisaccharide on H_2O_2 oxidation (see Table 1 for R_G values). The exact position of attachment of the rhamnose moiety to the gentiobiose is unknown. A quercetin triglycoside was reported in *Camellia sinensis* with two glucoses and a rhamnose, but the nature of the interglycosidic links was not determined [14].

The fourth new glycoside is isorhamnetin-3-*p*-coumaroyl glucoside. This is related to kaempferol-3-β-(6″-*p*-coumaroyl) glucoside isolated from the leaves of *Tribulus terrestris* [15], and also identified in the present study. Both acylated glycosides have characteristic UV spectra, and alkaline hydrolysis gave rise to *p*-coumaric acid and the corresponding 3-glucosides. Both are unaffected by β-glucosidase (see Table 1). Two kaempferol-*p*-coumaroylglucosides are reported in nature: tiliroside from *Tilea argentea* [16–18] and *Thymelea hirsuta* [19], and tribuloside from *Tribulus terrestris* [15]. In the present study, kaempferol 3-*p*-coumaroylglucoside was isolated from *Tribulus pentandrus*, *T. terrestris* and *Thymelea hirsuta*. All co-chromatographed and showed identical R_f values to those of kaempferol 3-*p*-coumaroylglucoside isolated from *Tilea argentea* [18]. (R_f in BAW 85, H_2O 5; lit. [18] BAW 88, H_2O 4.) Their UV data also agreed with that reported in the literature [15–19]. The acylated glucoside from all three plants afforded on H_2O_2 oxidation one and the same *p*-coumaroylglucose. The *p*-coumaroylglucose from all three plants had identical R_f values to those reported

Table 1. Chromatographic and hydrolytic data of flavonoid

Compound	Presence and relative quantity* *T. pentandrus*	*T. terrestris*	BAW	R_fs (×100)† H_2O	HOAc
Kaempferol (K)					
-3-glucoside	+	−	68	14	31
-7-glucoside	+	−	47	2	15
-3-gentiobioside	+ +	+	36	38	52
-3-rutinoside	−	+	48	31	52
-3-*p*-coumaroylglucoside	+	+	84	5	29
-3,7-diglucoside	+	−	35	50	64
-3-gentiobioside-7-glucoside	+ +	t	19	58	67
Quercetin (Q)					
-3-glucoside	+	+	54	13	32
-7-glucoside	+	−	29	1	7
-3-gentiobioside	+ + +	+ + +	32	26	44
-3-rutinoside	+	+ +	41	33	50
-3-gentiotrioside	+	+	28	42	61
-3-rhamnogentiobioside	−	+	27	54	65
-3,7-diglucoside	+	−	28	37	58
-3-gentiobioside-7-glucoside	+ +	+	8	44	58
-3-rutinoside-7-glucoside	+	−	22	50	66
Isorhamnetin (I)					
-3-glucoside	−	+	61	11	35
-3-gentiobioside	+	+	33	26	42
-3-rutinoside	−	+	45	22	46
-3-*p*-coumaroylglucoside	+	+	79	3	25
-3-gentiotrioside	−	t	13	—	49
-3,7-diglucoside	t	t	33	33	55
-3-gentiobioside-7-glucoside	+	+	17	60	70
-3-gentiotrioside-7-glucoside	−	t	14	52	65
Tricin (T)					
-7-diglucoside	t	−	33	0	10

*(+ + +)—Major, (+ +)—strong, (+)—weak, (t)—trace, (−)—absent.

†BAW—*n*-butanol–HOAc–H_2O, 4:1:5; H_2O—water; HOAc—15% acetic acid; PhOH—phenol–water, 4:1.

‡K—kaempferol, Q—quercetin, I—isorhamnetin, T—tricin, 3G—3-glucoside, 7G—7-glucosides, 37G—3,7-diglucoside, 3 Gent—3-gentiobioside, 3 Gentr—3-gentiotrioside, t—traces.

§RG values in BAW and BBPW (C_6H_6–*n*-butanol–pyridine–H_2O, 1:5:3:3, upper) respectively. Gentiobiose: 0.4, 0.41; gentriotriose: 0.32, 0.33; rutinose: 0.69, 0.76; rhamnogentiobiose: 0.52, 0.15.

‖Alkaline hydrolysis.

¶%See Experimental for R_f values.

by Harborne in *Tilea argentea* [18] (see Experimental). Also, enzymic hydrolysis with β-glucosidase of all samples isolated from *Tribulus pentandrus*, *T. terrestris* and *Thymelea hirsuta* failed to cleave the sugar moiety, which also agrees with the results of *Tilea argentea* [18]. The presence of isorhamnetin 3-*p*-coumaroylglucoside in *Tribulus* along with the corresponding kaempferol glycoside could have been overlooked by earlier workers [15]. This would explain the lower mp as well as the differences in other physical properties of 'tribuloside'. It would appear that tiliroside and tribuloside are one and the same, and only the former name (tiliroside) need be used, along with its physical properties [16–19].

Small amounts of two glycosides were isolated from *Tribulus terrestris*. They were identified as isorhamnetin-3-gentiotrioside and isorhamnetin-3-gentiotrioside-7-glucoside. Enzymic hydrolysis of the latter gave rise to the former 3-gentiotrioside. H_2O_2 oxidation of both compounds gave rise to a saccharide with R_G values (Table 1) which are comparable with those expected for gentiotrios. The UV data and R_f values (Tables 1 and 2) indicate that both glycosides are in agreement with the structures

glycosides from *Tribulus pentandrus* and *T. terrestris*

PhOH	Mild acid hydrolysis‡	Enzymic hydrolysis‡ (β-glucosidase)	H_2O_2 oxidation§
65	—	K	Glucose
57	—	K	—
41	K, K3G(t)	K, K3G (t)	Gentiobiose
58	K, K3G	*– ve*	Rutinose
80	K3G, *p*-coumaric‖	*– ve*	*p*-Coumaroylglucose¶
61	K, K7G	K, K3G	Glucose
36	K, K37G (t), K7G	K, K3Gent	Gentiobiose
48	—	Q	Glucose
28	—	Q	—
26	Q, Q3G (t)	Q, Q3G (t)	Gentiobiose
32	Q, Q3G	*– ve*	Rutinose
18	Q, Q3G (t), Q3Gent	—	Gentiotriose
27	Q, Q3G (t), Q3Gent	*– ve*	Rhamnogentiobiose
37	Q, Q7G	Q, Q3G	Glucose
14	Q, Q7G, Q37G (t)	Q, Q3Gent	Gentiobiose
29	Q, Q7G, Q37G	Q3-rutinoside	Rutinose
75	—	I	Glucose
52	I, I3G (t)	I, I3G (t)	Gentiobiose
65	I, I3G	*– ve*	Rutinose
87	I3G, *p*-coumaric.	*– ve*	*p*-Coumaroylglucose¶
—	I, I3G (t), I3Gent	—	Gentiotriose
70	I, I7G	I, I3G	Glucose
44	I, I7G, I37G (t)	I, I3Gent	Gentiobiose
50	I, I7G, I37G (t)	13Gent	Gentiotriose
91	T, T7G	T	—

assigned to them. The remaining glycosides are well-known, and their data are recorded in Tables 1–3.

DISCUSSION

According to Engler [20], the Zygophyllaceae is divided into seven subfamilies: Peganoideae, Chitonioideae, Tetradiclidoideae, Augeoideae, Zygophylloideae, Nitrarioideae and Balanitoideae. Hutchinson [21] considered the Zygophyllaceae as a homogeneous family, while Takhtajan [22] divided these taxa into four families, the Peganaceae V. Tiegh., Zygophyllaceae R.Br., Nitrariaceae Lindl. and Balanitaceae Endlich. The fifth subfamily, Zygophylloideae (sensu Engler) is further subdivided into two tribes: Zygophyllaeae Engl. and Tribuleae Engl. The tribe Tribuleae is comprised of five genera with close natural affinities [23]. Of these, *Neoluederitzia* Schinz., *Sisyndite* E. Mey. ex Sond. and Harv., and *Kelleronia* Schinz. are endemic to Africa, while *Tribulus* L. is widespread. *Kallstroemia* Scop, is endemic to Australia [23]. In a systematic study of Tribuleae, it was proposed to be treated as a distinct family: Tribulaceae Hadidi [1, 2].

A preliminary study of the flavonoid characters of

Table 2. UV data of flavonoid glycosides from *Tribulus pentandrus* and *T. terrestris*

Compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
Kaempferol -3-*p*-coumaroylglucoside	255	246	277	279	276	258
	268	278	312	312	317	270
	315	310sh	404	404	376	317
	360sh	375				362sh
		425sh				
-3-gentiobioside	266	275	276	276	276	256
	299sh	326	305	302	310	285sh
	352	400	352	352	389	344
			400	400		
-3-gentiobioside-7-glucoside	267	280	276	276	250sh	250sh
	330sh	305sh	300	300	268	267
	350	410	350	348	360sh	354
			400	399	400	
Quercetin -3-gentiobioside	258	274	275	270	270	262
	268sh	328	305sh	300sh	322	302sh
	363	414	332	362	390	380
			430	400		
-3-gentiotrioside	256	276	275	271	268	262
	267sh	325sh	305sh	303sh	320sh	370
	356	415	332sh	360	385	
			430	400		
-3-rhamnogentiobioside	256sh	278	273	268	270	262
	267	326sh	305sh	300sh	320sh	370
	350	417	332sh	360	380	
			428	400		
-3-gentiobioside-7-glucoside	256	249	275	270	258	262
	269sh	388	435	365	365	380
	358			400	420sh	
-3-rutinoside-7-glucoside	258	243	274	268	258	260
	268	276	305sh	302sh	265	370
	357	310sh	430	360	419sh	
		420		400		
Isorhamnetin -3-*p*-coumaroylglucoside	256	240	274	268sh	278	256
	271	283	305	276	315	270
	315	366	405	305	380	316
	360sh	420sh		405		365sh
-3-gentiobioside	255	284	268	267	274	258
	267sh	330	277sh	276sh	324	271
	356	420	305sh	305sh	408	360
			365	360		
			404	403		
-3-gentiotrioside	256	278	265sh	265sh	278	258
	272	332	280	278	320sh	271sh
			305sh	305sh		354
	350	420	344	340	410	
			410	410		
-3-gentiobioside-7-glucoside	255	250sh	270	269	257	257
	270sh	280	305sh	305sh	270sh	270sh
	352	430	364	364	368	361
			405	405	420	
-3-gentiotrioside-7-glucoside	255	248	272	270	255	255
	270sh	258sh	305sh	305sh	275	275
	353	280	362	362	368	360
		420	405	405	416	
Tricin -7-diglucoside	250	261	257	257	249	248
	269	280	282	282	265	268
	347	410	344	337	350	350
			373	370	426	

Table 3. NMR data of four flavonoid glycosides from *Tribulus pentandrus*

Compound	Flavonoid protons						Glucosyl protons		
	H-6	H-8	H-2′	H-3′	H-5′	H-6′	H_1-	Non-anomeric	Rhamnosyl
Kaempferol-3-gentiobioside	6.25*d*	6.48*d*	8.15*d*	7.00*d*	7.00*d*	8.15*d*	4.9–5.4 (2 protons)	3–4 (12 protons)	—
Quercetin-3-gentiobioside	6.29*d*	6.49*d*	7.69*dd*	—	6.94*d*	7.69*dd*	4.5–5.4 (2 protons)	3–4 (12 protons)	—
Quercetin-3-gentiobioside-7-glucoside	6.27*d*	6.45*d*	7.69*dd*	—	6.92*d*	7.69*dd*	4.7–5.4 (3 protons)	3–4 (18 protons)	—
Quercetin-3-rutinoside	6.27*d*	6.45*d*	7.6*dd*	—	6.92*d*	7.6*dd*	4.46–5.4 (2 protons)	3–4 (10 protons)	1 (3 protons)

Spectra were recorded in DMSO on a Varian EM-360 60 spectrometer. Values are given in ppm (δ scale) relative to TMS as an int. standard; *d* (doublet), *dd* (double doublet).

Zygophyllaceae was carried out [11]. This indicated that the glycosylation patterns within the family might play an important role in the chemosystematics. The present study of the chemistry of *Tribulus pentandrus* and *T. terrestris* indicates that a difference exists between both species, which in turn belong to two different sections: Alata and Terrestris respectively [1]. In both species quercetin 3-gentiobioside is the major glycoside. However, the second major glycoside in *T. terrestris* is quercetin 3-rutinoside, while in *T. pentandrus* kaempferol 3-gentiobioside and the 3-gentiobioside-7-glucosides of kaempferol and quercetin are the second major glycosides. A second difference between the glycosides of both species is the replacement of kaempferol glycosides in *T. pentandrus* by a larger variation of isorhamnetin glycosides in *T. terrestris*.

The rutinosides represent the major glycosidic patterns in members of Zygophyllaceae previously examined [11]. The fact that gentiobiosides are the major glycosidic patterns is *Tribulus* species, along with other morphological characters [1, 2] might favour its separation into a separate family. Other *Tribulus* species along with representatives of the remaining species belonging to Tribulaceae Hadidi are presently under investigation.

EXPERIMENTAL

Material. Fresh material of *Tribulus pentandrus* Forssk. and *T. terrestris* L. were collected from the suburbs of Cairo. Samples were identified by Mrs Hasnaa A. Hosni, Department of Botany, Cairo University. Voucher specimens are deposited in the Herbarium, Cairo University.

Methods. Samples were extracted with 70% EtOH, extracts evaporated under red. pres. followed by CC on polyamide. Fractions were further fractionated using elution techniques. Pure compounds were investigated and their structures determined according to standard methods [12, 24]. Mild acid hydrolysis was carried out with 0.1 N HCl, enzymic hydrolysis with β-glucosidase (Fluka) using an acetate buffer (pH 5) and H_2O_2 oxidation according to the method of ref. [25].

Melting points. Kaempferol 3-gentiobioside: 202–205° (lit. 203–204° [26]), kaempferol 3-rutinoside: 220–221° (lit. 220–222° [15]), kaempferol 3-gentiobioside-7-glucoside: 220°, quercetin 3-gentiobioside: 200–202° (lit. 202–204° [26]), quercetin 3-rutinoside: 190–192° (lit. 188–190° [27]), quercetin 3-gentiobioside-7-glucoside: 225°, isorhamnetin 3-rutinoside: 176–177° (lit. 174–177° [28]).

p-*Coumaroylglucose.* H_2O_2 oxidation of both acylated kaempferol and isorhamnetin glucosides gave rise to *p*-coumaroylglucose. R_f values of *p*-coumaroylglucose and *p*-coumaric acid respectively in BAW: 55 and 85; PhOH: 75 and 64; H_2O: 67, 83 and 50, 59.

TLC. The following R_f values were obtained on Si gel in C_6H_6–MeOH (4:1) and toluene: HCO_2Et–HCO_2H (5:4:1), respectively. Kaempferol 3-glucoside: 0.2, 0.16; kaempferol 3-*p*-coumaroylglucoside: 0.23, 0.28; isorhamnetin 3-*p*-coumaroylglucoside: 0.25, 0.28.

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