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THE FLAVONOIDS OF *TETRAGONOTHECA* (COMPOSITAE)

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Key Word Index—*Tetragonotheca*; Compositae; Heliantheae; 6-hydroxyflavonols; flavonol 7-*O*-glycosides.

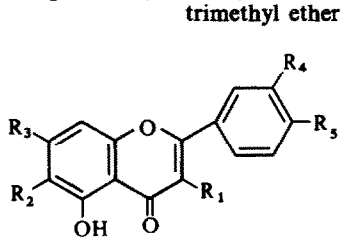
Abstract—Fifteen flavonols, five aglycones and ten glucosides were isolated from the four species of *Tetragonotheca*, *T. repanda*, *T. helianthoides*, *T. texana* and *T. ludoviciana*. Included among the isolated flavonols are four previously unreported 7-*O*-glucosides, 6-hydroxykaempferol 7-*O*-glucoside, 6-hydroxykaempferol 6-methyl ether 7-*O*-glucoside, quercetagetin 6,3′-dimethyl ether 7-*O*-glucoside and quercetagetin 3,6-dimethyl ether 7-*O*-glucoside.

INTRODUCTION

Tetragonotheca L. is a small, asteraceous genus of four North American species: *T. helianthoides* L., southeastern United States; *T. ludoviciana* (T. & G.) Gray, eastern Texas, Louisiana and Oklahoma; *T. texana* (Gray) Englem. & Gray, Edwards Plateau region and Rio Grande Plains of Texas and northeastern Mexico; *T. repanda* (Buckl.) Small, southeastern Texas. The genus, which belongs to the tribe Heliantheae, is distinctive morphologically in that the outer phyllaries are relatively large and conspicuous and always number four. The flavonoids of the genus add to its distinctive nature; as we report here, the group is characterized by the production of C_6 oxygenated flavonols, four of which are previously unreported as natural products.

RESULTS AND DISCUSSION

Tetragonotheca accumulates 19 non-floral flavonoids, of which 15 (Table 1) have been isolated and chemically characterized (Fig. 1); in addition, two of the remaining four have been partially characterized and are designated A and B in Tables 1 and 2. Of the identified compounds, five are known aglycones, kaempferol 3-methyl ether (**1**), quercetin 3-methyl ether (**2**), quercetagetin 6-methyl ether (**3**), quercetagetin 3,6-dimethyl ether (**4**), quercetagetin 3,6,4′-trimethyl ether (**5**); six are known glycosides (although this is only the second report of compound **8**), 6-hydroxykaempferol 3,6-dimethyl ether 7-*O*-glucoside (**8**), quercetagetin 7-*O*-glucoside (**9**), quercetagetin 6-methyl ether 7-*O*-glucoside (**10**), quercetagetin 3,6,3′-trimethyl ether 7-*O*-glucoside (**13**), quercetagetin 3,6,4′-



1 R_1 = OMe, R_2 = H, R_3 = OH, R_4 = H, R_5 = OH (kaempferol 3-methyl ether)
2 R_1 = OMe, R_2 = H, R_3 = OH, R_4 = OH, R_5 = OH (quercetin 3-methyl ether)
3 R_1 = OMe, R_2 = OMe, R_3 = OH, R_4 = OH, R_5 = OH (quercetagetin 3,6-dimethyl ether; axillarin)
4 R_1 = OMe, R_2 = OMe, R_3 = OH, R_4 = OH, R_5 = OMe (quercetagetin 3,6,4′-trimethyl ether; centaureidin)
5 R_1 = OH, R_2 = OMe, R_3 = OH, R_4 = OH, R_5 = OH (quercetagetin 6-methyl ether; patuletin)
6 R_1 = OH, R_2 = OH, R_3 = O-Glc, R_4 = H, R_5 = OH (6-hydroxykaempferol 7-*O*-glucoside)
7 R_1 = OH, R_2 = OMe, R_3 = O-Glc, R_4 = H, R_5 = OH (6-hydroxykaempferol 6-methyl ether 7-*O*-glucoside)
8 R_1 = OMe, R_2 = OMe, R_3 = O-Glc, R_4 = H, R_5 = OH (6-hydroxykaempferol 3,6-dimethyl ether 7-*O*-glucoside)
9 R_1 = OH, R_2 = OH, R_3 = O-Glc, R_4 = OH, R_5 = OH (quercetagetin 7-*O*-glucoside)
10 R_1 = OH, R_2 = OMe, R_3 = O-Glc, R_4 = OH, R_5 = OH (quercetagetin 6-methyl ether 7-*O*-glucoside; patulitrin)
11 R_1 = OH, R_2 = OMe, R_3 = O-Glc, R_4 = OMe, R_5 = OH (quercetagetin 6,3′-dimethyl ether 7-*O*-glucoside; spinacetin 7-*O*-glucoside)
12 R_1 = OMe, R_2 = OMe, R_3 = O-Glc, R_4 = OH, R_5 = OH (quercetagetin 3,6-dimethyl ether 7-*O*-glucoside; axillarin 7-*O*-glucoside)
13 R_1 = OMe, R_2 = OMe, R_3 = O-Glc, R_4 = OMe, R_5 = OH (quercetagetin 3,6,3′-trimethyl ether 7-*O*-glucoside; jacein)
14 R_1 = OMe, R_2 = OMe, R_3 = O-Glc, R_4 = OH, R_5 = OMe (quercetagetin 3,6,4′-trimethyl ether 7-*O*-glucoside; centaurein)
15 R_1 = O-Glc, R_2 = H, R_3 = OH, R_4 = OMe, R_5 = OH (isorhamnetin 3-*O*-glucoside)

Fig. 1. Structure of elucidated flavonols of *Tetragonotheca*.

Table 1. Distribution of flavonols in *Tetragonotheca*

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	A	B
T. repanda		+	+		+					+		+					
T. helianthoides		+	+	+										+			
T. texana	+	+				+			+							+	+
T. ludoviciana	+	+	+				+	+			+	+	+		+		

Table 2. Chromatographic properties and UV absorption maxima (nm) for *Tetragonotheca* flavonoids

Compound	MeOH	NaOMe*	$AlCl_3$†	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3	R_f values‡ TBA	R_f values‡ HOAc	Color§ UV	Color§ +NH_3
1	268 299sh 349	275 325 396	277 305 350 399	277 305 347 399	277 310 389	268 303sh 351	0.85	0.21	p	g
2	257 270sh 295sh 357	272 327 407	277 305 440	271 305 365 406	272 324 385	261 295sh 377	0.76	0.14	p	y
3	259 272sh 292sh 353	273 327 408	279 309 441	271 304 317sh 390	273 329 399	266 299sh 377	0.72	0.18	p	y
4	256 272 350	274 307sh 388	268 280sh 302sh 380 395sh	265 280 365	274 376	256 272sh 352	0.80	0.17	p	p
5	257 273 372	335 (dec)	275 459	266 305sh 370sh 430	275 335 400 (dec)	263 389	0.69	0.05	y	o
6	238 257 276 350	248 290 400 (dec- rapid)	242 264sh 290 386 456sh	239 268 281sh 381 421	285 382 (dec- rapid)	255sh 281 345 382sh	0.32	0.08	dp	dp
7	256 269 335sh 366	233sh 253 267 420 (dec)	232 267 300sh 368sh 422	235sh 270 303sh 368 426	255 334 412 (dec)	257 268 338sh 367	0.57	0.22	y	yg
8	271 339	272 398	279 302sh 368 402sh	280 301sh 358 405sh	269 398	269 346	0.72	0.54	p	g
9	256 272sh 353	245 296 372 (dec)	281 441	271 388 414sh	392 380 (dec)	266 368	0.06	0.04	dp	br-p
10	257 274sh 371	270 298sh 438 (dec)	274 344 458	266 372sh 428	258 340sh 408 (dec)	261 388	0.24	0.15	y	o
11	256 272sh 366	265 415 (dec)	270 274sh 420	268 372sh 420	259 412 (dec)	256 369	0.47	0.16	y	y
12	260 272sh 355	272 416	277 442	265 280sh 374 401sh	267 415	265 380	0.59	0.40	p	y

Table 2.—*Continued*

Compound	MeOH	NaOMe*	$AlCl_3$†	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3	R_f values‡ TBA	R_f values‡ HOAc	Color§ UV	Color§ $^+NH_3$
13	255 271 344	246 271 412	270 281sh 302sh 382	266 281sh 300sh 367 401sh	265 413	257 271sh 351	0.69	0.47	p	br-y
14	258 274sh 350	273 382	271 301sh 379	269 279 368	260 270 349	259 272sh 350	0.64	0.22	p	p
15	255 270sh 351	272 330 415	268 301 361sh 402	267 276sh 302sh 359 399	274 325 407	256 270sh 300sh 359	0.66	0.43	p	yg
A	259 278 348	263 296 390 (dec)	280 296sh 434	240sh 266 288 376	268 387 (dec)	267 286sh 365	0.31	0.19	dp	gp
B	272 341	246 309 380 (dec)	240 302 375	239 301 372	299 367 (dec)	—	0.53	0.31	dp	br-p

* dec = decomposition.
† sh = shoulder.
‡ TBA = tertiary butanol–glacial acetic acid–H_2O, 3:1:1 v/v; HOAc = 15% acetic acid.
§ p = purple, g = green, y = yellow, o = orange, d = dark, br = brown.

Table 3. NMR spectral data for ten *Tetragonotheca* flavonols*

Compound	$H_{2'}$	$H_{6'}$	$H_{3'}$	$H_{5'}$	H_6	H_8	Glucosyl $H_{1''}$	Glucosyl Non-anomeric protons	OMe in CCl_4 (ΔC_6D_6) 3-OMe	6-OMe	3'-OMe
1	8.03*d* (*J* = 9)	8.03*d* (*J* = 9)	6.89*d* (*J* = 9)	6.89*d* (*J* = 9)	6.18*d* (*J* = 2)	6.49*d* (*J* = 2)	—	—	3.87	—	—
2	7.62(*s*)	7.47(*d*) (*J* = 2)	—	6.87*d* (*J* = 9)	6.15*d* (*J* = 2)	6.45*d* (*J* = 2)	—	—	3.88	—	—
3	7.60(*s*)	7.48(*d*) (*J* = 2)	—	6.84*d* (*J* = 9)	—	6.49	—	—	3.85	3.72	—
6	8.03*d* (*J* = 9)	8.03*d* (*J* = 9)	6.86*d* (*J* = 9)	6.86*d* (*J* = 9)	—	6.60	5.20–4.90*m*	3.90–3.50*m*	—	—	—
7	8.04*d* (*J* = 9)	8.04*d* (*J* = 9)	6.86*d* (*J* = 9)	6.86*d* (*J* = 9)	—	6.66	5.12–4.90*m*	3.80–3.50*m*	—	3.77 (Δ+0.03)	—
8	7.96*d* (*J* = 9)	7.96*d* (*J* = 9)	6.86*d* (*J* = 9)	6.86*d* (*J* = 9)	—	6.64	5.08–4.94*m*	3.72–3.50*m*	3.76	3.86	*m*
9	7.59(*s*)	7.65(*m*)	—	6.81*d* (*J* = 9)	—	6.58	5.18–4.95*m*	3.88–3.39*m*	—	—	—
11	7.72*d* (*J* = 2)	7.52*dd* (*J* = 2) (*J* = 9)	—	6.82*d* (*J* = 9)	—	6.62	5.12–4.88*m*	3.80–3.40*m*	—	3.75 (Δ+0.03)	3.87 (Δ+0.27)
12	7.68(*s*)	7.60(*dd*) (*J* = 2) (*J* = 9)	—	6.88*d* (*J* = 9)	—	6.67	5.20–4.92*m*	3.90–3.40*m*	3.90 (Δ+0.06)	3.79 (Δ+0.05)	—
13	7.66*d* (*J* = 2)	7.51*dd* (*J* = 2) (*J* = 9)	—	6.86*d* (*J* = 9)	—	6.63	5.22–4.98*m*	3.72–3.50*m*	3.87	3.73	3.91

* Chemical shifts are given in ppm (δ scale) relative to TMS as internal standard; coupling constants are given in Hz. Spectra were recorded for trimethylsilyl ethers on a Varian EM-360 spectrometer. Signals are singlets unless noted: *d* = doublet, *dd* = double doublet, *m* = multiplet, (*s*) = single peak of overlapping signals, (*d*) = 1/2 proton doublet with downfield signal overlapping $H_{2''}$, (*dd*) = part of downfield signal overlapping $H_{2''}$, (*m*) = signal broad with one peak overlapping $H_{2''}$.

trimethyl ether 7-*O*-glucoside (**14**), isorhamnetin 3-*O*-glucoside (**15**); and four are new glycosides, 6-hydroxykaempferol 7-*O*-glucoside (**6**), 6-hydroxykaempferol 6-methyl ether 7-*O*-glucoside (**7**), quercetagetin, 6,3′-dimethyl ether 7-*O*-glucoside (**11**), quercetagetin 3,6-dimethyl ether 7-*O*-glucoside (**12**). That compounds **6**, **7**, **11** and **12** were flavonols was indicated by their UV absorption (Table 2) (Band I MeOH at 350 nm or higher [1]) and in the case of **7** and **11**, their color on PC (Table 2) over UV light (366 nm). That **7**, **11** and **12** each possessed a 4′-hydroxyl group was confirmed by UV (Band I NaOMe bathochromic shift of 40–60 nm and increase in intensity compared with Band I in MeOH [1]). Compound **6** exhibited a NaOMe Band I shift in accord with a free 4′-hydroxyl group but decomposed so rapidly that no increase in intensity of absorption could be detected. That each compound lacked a free 7-hydroxyl group was indicated by the absence of a Band III absorption in NaOMe (Table 2) [2] and the absence of a significant Band II NaOAc bathochromic shift (Table 2) [1]. The color of each compound on PC in UV light (Table 2) confirmed each had a free 5-hydroxyl group.

The NMR spectra of the trimethylsilyl ethers of **6**, **7**, **11** and **12** indicated a single sugar moiety present in each compound (Table 3); the anomeric sugar proton in all spectra occurred between δ 4.88–5.20 (Table 3), ruling out 3-glycosylation and suggesting 7-glycosylation [1]. GLC of the trimethylsilyl ethers of the acid-hydrolysed sugars of all compounds yielded only glucose.

The mass spectrum of the perdeuteriomethylated (PDM) derivative of **12** showed a molecular ion at m/e = 627 (Table 4), consistent with a hexaoxygenated, mono-*O*-glucoside with two *O*-methyl groups. UV spectral data (Table 2) indicated **12** was 3-*O*-substituted, possessed a 3′, 4′ *ortho*-dihydroxyl system (hypsochromic shift of 68 nm in Band I $AlCl_3$–HCl compared to Band I in $AlCl_3$ and a 25 nm Band I bathochromic shift in NaOAc–H_3BO_3 compared to Band I in MeOH) and was 6-methoxylated (Band I bathochromic shift of only 19 nm in $AlCl_3$–HCl compared with Band I in MeOH [3]). The NMR spectrum (Table 3) exhibited signals for four aromatic protons and two *O*-methyl groups in addition to the glucose signals. Coupling relationships clearly allowed assignment of three protons to the B-ring; a slightly broadened singlet at δ 7.68, $H_{2'}$; a double doublet at δ 7.60, J = 2, J = 9, $H_{6'}$; a doublet at δ 6.88, J = 9, $H_{5'}$; and the fourth aromatic proton, a singlet at δ 6.67, to the C-ring or the A-ring. An *O*-methyl group at C_7 was ruled out due to the small magnitude of the benzene-induced shift in the *O*-methyl resonances [4], Δ + 0.06 and 0.05 (Table 3). Since the singlet at δ 6.67 is in the range of the chemical shift for a C_8 proton in 7-*O*-glucosylated flavonols [1], one *O*-methyl group is assigned to C_6, one to C_3 and the glucosyl moiety to C_7. UV analysis of the aglucone, recovered after acid hydrolysis of the glucoside, indicated a free hydroxyl group at C_7 (NaOMe Band III absorption at 330 nm and Band II bathochromic shift of 14 nm in NaOAc compared to Band II in MeOH [1]) confirming the position of glucosylation. Therefore, **12** must be quercetagetin 3,6-dimethyl ether 7-*O*-glucoside.

The mass spectrum of the PDM derivative of **11** gave a molecular ion at m/e = 627 (Table 4), again consistent with a hexaoxygenated, mono-*O*-glucoside with two *O*-methyl groups. The NMR spectrum of the glucoside (Table 3) exhibited signals for four aromatic protons and two *O*-methyl groups. Coupling among three protons allowed their assignment to the B-ring positions $C_{2'}$, $C_{5'}$, $C_{6'}$ (Table 3), and the fourth aromatic proton, a singlet at δ 6.62, to the A-ring, since the compound has a free 3-hydroxyl group (yellow color on PC in UV and Band I UV absorption at 366 nm in MeOH (Table 2)). Benzene-induced shifts in the *O*-methyl resonances (Table 3) allowed one *O*-methyl to be assigned to $C_{3'}$, Δ + 0.27 (supported by UV Band II long wavelength shoulder in MeOH and lack of a hypsochromic shift in Band I in $AlCl_3$–HCl compared to Band I in $AlCl_3$ (Table 2)); the small induced shift of the remaining *O*-methyl group, Δ + 0.03, and the magnitude of the chemical shift of the single A-ring proton, δ 6.62, as previously discussed, allows assignment of the *O*-methyl to C_6 and the *O*-glucosyl moiety to C_7. (UV analysis of the aglucone confirmed the position of sugar attachment since a NaOMe Band III absorption at 333 nm and a bathochromic shift of 13 nm in Band II NaOAc compared with Band II MeOH indicated a free C_7 hydroxyl group.) Compound **11** is, therefore, quercetagetin 6,3′-dimethyl ether 7-*O*-glucoside.

The mass spectrum (PDM) of **7** gave a molecular ion at m/e = 597 (Table 4), indicating a pentaoxygenated mono-*O*-glucoside with one *O*-methyl group. The NMR spectrum (Table 3) indicated five aromatic protons, four with the typical pattern for a $C_{4'}$ hydroxylated B-ring, two doublets, δ 8.04, J = 9, $H_{2'}$, $H_{6'}$ and δ 6.86, J = 9, $H_{3'}$, $H_{5'}$; and one singlet, δ 6.66, assignable to the A-ring. The small benzene shift of the *O*-methyl resonance, Δ + 0.03 (Table 3), and the chemical shift of the A-ring proton allowed assignment of the *O*-methyl to C_6 and the *O*-glucosyl function to C_7, assignments which were supported by UV analysis of the aglucone. The natural product is 6-hydroxykaempferol 6-methyl ether 7-*O*-glucoside.

The mass spectrum (PDM) of **6** gave a molecular ion at m/e = 600 (Table 4), for a pentaoxygenated mono-*O*-glucoside. The NMR spectrum (Table 3) indicated five aromatic protons, four distributed in the typical two doublet pattern for a single $C_{4'}$-hydroxylated B-ring and a singlet at δ 6.60 (the C_8 proton region in 7-*O*-glucosylated flavonols). That the singlet at δ 6.60 was indeed the C_8 proton was confirmed by the 0.15 ppm upfield shift of this signal in the NMR spectrum of the partial trimethylsilyl ether with the C_5 hydroxyl free (In such instances C_6 protons show no significant shift and flavone C_3 protons shift downfield 0.15 ppm [1, 5]). Additionally, permethylated derivatives of **6** and **7** exhibited identical mass spectral fragmentation patterns. Therefore, **6** is identified as 6-hydroxykaempferol 7-*O*-glucoside.

Two additional glucosides have been isolated from *T. texana*. Both have color and UV spectra characteristic for 6-hydroxylated flavonols (Table 2). Both yield glucose on hydrolysis and the glucose is not at C_3. One is a quercetagetin derivative, the other a derivative of 6-hydroxykaempferol. Additional data are required for their full characterization.

The UV spectra of 6-hydroxykaempferol 7-*O*-glucoside (Table 2) are atypical of 3-hydroxylated flavonols. MeOH Band I absorption at 350 nm is considerably lower than one would expect (compare with **7** (Table 2); kaempferol 7-*O*-glucoside has Band I in MeOH at 366 nm). The $AlCl_3$ absorption maximum for Band I is also less than expected (compare with **7**; kaempferol 7-*O*-glucoside at 422 nm). This latter phenomenon may prove

Table 4. Mass spectral fragmentation data for four *Tetragonotheca* perdeuteriomethylated 6-hydroxyflavonol 7-*O*-glucosides

Compound	M^+	M − 1	M − 16	M − 17	Other major fragments*
6	600 † (42.7)‡	599 (7.7)	584 (13)	583 (26.6)	371 (31.7), 370 (68.6), 369 (46.7), 353 (57.1), 352 (100), 341 (21.0)
7	597 (67.1)	596 (14.6)	581 (9.1)	580 (17.9)	368 (21.1), 367 (55.3), 366 (39.2), 352 (35.5), 351 (28.8), 350 (55.6), 349 (62.2)
11	627 (59.9)	626 (4.2)	611 (2.3)	610 (6.5)	397 (46.7), 396 (21.1), 382 (26.9), 381 (13.4), 380 (28.2), 379 (42.8), 368 (15.2)
12	627 (63.9)	626 (10.8)	611 (6.9)	610 (13.9)	398 (32.6), 397 (62.6), 396 (40.7), 382 (32.5), 381 (21.3), 380 (49.6), 379 (66.1), 368 (23.6)

* The spectrum of each compound exhibited glucosyl fragments at *m/e* 230, 196, 161, 107, 81, 74; in **7**, **11** and **12**, 107 was the base peak.

† Numbers denote observed *m/e* values for fragment ions.

‡ Numbers in parentheses denote relative intensities of observed ion.

to be characteristic of 3,6-dihydroxylated flavonols since similar observations have been recorded for quercetagetin [6], quercetagetin 3′-methyl ether [7] and 6-hydroxykaempferol [8].

Smith *et al.* [8] reported a Band I in MeOH absorption for 6-hydroxykaempferol (obtained by demethylation of eupalitin) at 360 nm, again less than expected for a 3-hydroxylated flavonol (kaempferol gives a Band I at 367 nm). Our sample, obtained by acid hydrolysis of **6**, showed a Band I absorption in MeOH of 352 nm. These discrepancies indicate the compound is unstable.

We also would point out and emphasize the utility of the chemical shift of the A-ring proton(s) in determining 7-*O*-glucosylation, as indicated, but not emphasized, by Mabry *et al.* [1] and Markham and Mabry [9]. The C_8 protons in the NMR spectra of all *Tetragonotheca* 7-*O*-glucosides appear between δ 6.58–6.67, as expected for C_8 protons in 7-*O*-glucosylated flavonols [1, 9]. Bohm *et al.* [10] recently reported NMR data for a number of flavonol C_6- and $C_{4'}$-*O*-glucosides; in all reported compounds the C_8 proton appeared at δ 6.24–6.29, clearly differentiating the 6- and 4′-*O*-glucosides from 7-*O*-glucosides. Conversely, assignment of methoxyl groups in flavonol 7-*O*-glucosides, even those with free C_3-hydroxyl groups, is facilitated by noting the magnitude of A-ring proton chemical shifts.

EXPERIMENTAL

Voucher specimens for *T. texana* (Bacon *1653* collected from U.S.A.: Texas: Travis Co.) and *T. ludoviciana* (Bacon & Bragg *1652*, collected from U.S.A.: Texas: Tarrant Co.) are on deposit in the herbarium of The University of Texas at Arlington; vouchers for *T. repanda* (Urbatsch *2460*, collected from U.S.A.: Texas: Brooks Co.) and *T. helianthoides* (Urbatsch *2750* collected from U.S.A.: Mississippi: Lamar Co.) are deposited in the herbarium of Louisiana State University. Air-dried leaves of each species were extracted overnight with $CHCl_3$–EtOAc (70:30 v/v), followed by a 24 hr extraction with 85% MeOH. The $CHCl_3$–EtOAc fraction, containing aglycones, was concd to a small vol. and chromatographed over a polyamide column (4.5 cm i.d.). Elution began with 30% EtOAc in $CHCl_3$ and continued until bands, detected with UV light (366 nm), ceased movement. Remaining compounds were eluted with $CHCl_3$–MeOH–MeCOEt (12:3:1). Collected bands were run 2D on PC using solvents as listed in Table 2. Heterogeneous fractions were made homogeneous by additional CC, using smaller columns (2.5 cm i.d.) and MeOH–$CHCl_3$ mixtures as solvents or by PC in 1D on Whatman 3MM paper. Similar procedures were used on the 85% MeOH extracts except that various mixtures of MeOH–$CHCl_3$ or 100% MeOH were used as CC solvents. All spectral methods and sugar analyses were those as presented by ref. [1].

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