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FLAVONOIDS OF *POLYGONUM SENEGALENSE* PART III: ISOLATION OF DIHYDROCHALCONE GLUCOSIDE AND QUERCETIN GLYCOSIDES

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ABSTRACT: The internal tissue flavonoids of *P. senegalense* are glycosides, based mostly on quercetin. Chromatography on silica gel and Sephadex LH-20 of the hydrophilic extract resulted in the isolation and characterizationof quercetin-3-ß-*O*-galactoside(**1**), quercetin-3-ß-*O*-glucoside (**2**), quercetin-3-(2"-galloylglucoside) (**3**) and a new compound 2'-glucosyl-4'-hydroxy-6'-methoxydihydrochalcone (or uvangolatin-2'-ß-*O*-glucoside) (**4**).

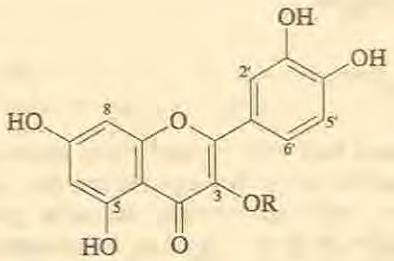
INTRODUCTION

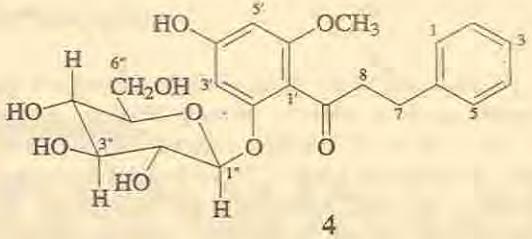
Polygonum senegalense Meisn (Polygonaceae) is a highland East African weed whose flavonoid chemistry we have looked at in some detail during the last few years. We reported that the plant has two types of flavonoids: external exudate flavonoid aglycones covering the aerial parts including the flower heads [1,2] and internal tissue glycosides which were based on the widespread aglycones, quercetin, kaempferol and luteolin [2]. The external exudate flavonoids have locust anti-feedant [1] as well as mosquito larvicidal activity [3]. The latter activity is also exhibited by quercetin.

Twelve external exudate flavonoids have been reported: 2'-hydroxy-3',6'-dimethoxychalcone, 2',4'-dihydroxy-3',6'-dimethoxychalcone, 2',6'-dihydroxy-3',4'-dimethoxychalcone, 2',6'-dihydroxy-4'-methoxydihydrochalcone, 2',6'-dihydroxy-4'-methoxychalcone, 2',4'-dihydroxy-6'-methoxychalcone, 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5,8-dimethoxyflavanone, 7-hydroxy-5-methoxyflavone, 3,7-dihydroxy-5,8-dimethoxyflavanone, 2',4'-dihydroxy-6'-methoxydihydrochalcone and 3',6'-dihydroxy-2',4',5'-trimethoxychalcone [1,2]. The fact that external flavonoids of *P. senegalense* are non-polar compared to the internal tissue forms is quite intriguing. It is interesting to reflect on the biosynthesis of these non-polar flavonoids. There are two possibilities for this process: formation directly from the 2',4',6'-trihydroxychalconeintermediate, further hydroxylation and methylation or transformation of the existing internal tissue flavonoids like quercetin. The former process is more likely but the latter cannot be ruled out. To lay ground for consideration of the biosynthesis, it was necessary to conduct a detailed analysis of the internal tissue flavonoids, a process which could lead to isolation of intermediate structures. In this paper we report the characterization of three quercetin glycosides and one new dihydrochalcone glucoside from the hydrophilic extract of the aerial parts of *P. senegalense*.

RESULTS AND DISCUSSION

The flavonoid aglycones, quercetin, kaempferol and luteolin, were identified by comparison with authentic compounds as already described [2]. Compound **1** on hydrolysis gave quercetin and a sugar group which was identified as galactose using paper chromatography against a standard. Even though the EI-MS of compound **1** had a major ion peak at *m/z* 302, the FAB-MS showed a molecular ion peak at *m/z* 464 suggesting that there is rapid loss of a galactosyl moiety from the molecular ion under EI conditions. The anomeric proton signal at δ 5.37 (*d*, *J* = 7.69 Hz) indicated that the galactose unit was attached to the flavonoid aglycone by a ß-linkage [4] and that it was at the C-3 [5]. The existence of the galactosyl moiety was also confirmed by the chemical shift and splitting pattern in the sugar region of the ^{1}H NMR spectrum (Table 1) [6]. The compound was assigned structure **1**, quercetin-3-ß-*O*-galactoside, and had a melting point identical with that reported for the same compound isolated from *Rumex acetosa* [7].





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1 R = β-galactosyl

2 R = β-glucosyl

3 R = β-(2"-galloylglucosyl)

The aglycone of compound **2** was identical to that of **1**. However, the sugar group was clearly glucose as evidenced by co-paper chromatography with standard glucose. That it was quercetin-3-ß-*O*-glucoside was confirmed by comparing the melting point and spectroscopic data with those reported for the same compound isolated from *Erica cinera* [6]. This structure is also supported by the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2).

The major glycoside of *P. senegalense* is quercetin-3-(2"-galloylglucoside) (**3**) which had been isolated and characterized from the same plant by Dossaji and Kubo [8]. We have now generated the ^{13}C NMR data for the compound (Table 2).

The most interesting glycoside isolated from *P. senegalense* was assigned the structure 2'-glucosyl-4'-hydroxy-6'-methoxydihydrochalcone (**4**). The aglycone of the compound, 2',4'-dihydroxy-6'-methoxydihydrochalcone(uvangolatin) was isolated earlier from the same plant [2]. Uvangolatin was also obtained from *Uvaria angolensis* [9]. The fact that there is no shift in peak positions in the UV spectrum of **4** on addition of $AlCl_3$ (but observed with uvangolatin) suggested that the glycosylation and methoxylation were at C-2' and 6'. Band I of the UV spectrum was shifted when sodium acetate was added to the methanol solution which confirmed the presence of a 4'-hydroxyl group. The sugar group was confirmed to be glucose based on the ^{1}H NMR spectrum and by comparing the sugar obtained after acid hydrolysis with standard glucose on paper chromatography. The attachment of the glucose unit by a ß-bond is shown by the fact that the anomeric proton absorbs at δ 4.86 (J = 7.76 Hz) in the ^{1}H NMR spectrum. It is interesting to note that this compound could be found accumulated in the vacuoles of *P. senegalense;* it may be the final intermediate in the transformation of any of the polar internal tissue flavonoids to uvangolatin or the final intermediate in the biosynthesis of this flavonoid directly from cinnamic acid.

Table 1. ^{1}H NMR data of compounds **1-4**.

H	1	2	3	4
1	-	-	-	7.18 - 7.30 *m*
2	-	-	-	"
3	-	-	-	"
4	-	-	-	"
5	-	-	-	"
6	6.19 *d*, J = 1.98	6.20 *d*, J = 2.07	6.17 *d*, J = 1.96	-
7	-	-	-	2.87 *t*
8	6.39 *d*, J = 1.98	6.40 *d*, J = 2.07	6.34 *d*, J = 1.96	3.22 *t*
2'	7.51 *d*, J = 2.21	7.53 *d* J = 2.24	7.77 *d*, J = 2.21	-
3'	-	-	-	6.16 *d*, J = 1.91
5'	6.80 *d*, J = 8.50	6.81 *d*, J = 8.50	6.81 *d*, J = 8.50	6.32 *d*, J = 1.91
6'	7.51 *d*, J = 8.50	7.67 *d*, J = 2.24	7.50 *dd*, J = 8.50, 2.21	-
6'-OCH_3	-	-	-	3.71 *s*
1"	5.37 *d*, J = 7.69	5.37 *d*, J = 7.66	5.10 *d*, J = 7.87	4.80 *d*, J = 7.76
2"	3.55 *d*, J = 9.27	3.30 - 3.57 *m*	3.40 - 3.80 *m*	3.30 - 3.69 *m*
3"	3.80 *m*	-	"	"
4"	3.63 *m*	3.30 - 3.57 *m*	"	"
5"	3.30 *m*	3.30 - 3.57 *m*	"	"
6"	3.44 *d*, J = 5.93	3.30 - 3.57 *m*	"	"
2'''	-	-	6.88 *s*	-
6'''	-	-	6.88 *s*	-

Table 2. ^{13}C NMR data of compounds **1-4**.

C	1	2	3	4
1	-	-	-	129.4
2	156.2	158.7	158.3	129.5
3	133.5	135.8	135.7	126.8
4	177.4	179.5	179.5	129.7
5	161.2	163.0	162.1	126.8
6	98.6	94.7	99.9	129.5
7	164.0	166.2	168.0	31.2
8	93.4	94.7	94.8	47.5
9	156.2	158.7	158.3	206.4
10	103.9	105.6	105.5	-
1'	121.1	122.9	123.0	114.1
2'	115.9	116.1	116.1	162.3
3'	144.7	145.8	145.7	97.0
4'	148.4	150.0	149.9	157.8
5'	115.9	117.8	117.8	94.7
6'	121.0	123.0	123.0	162.3
1"	101.8	105.6	105.5	103.1
2"	71.2	73.2	74.6	74.8
3"	73.2	75.1	74.6	78.4
4"	67.9	70.0	71.6	71.2
5"	75.8	77.2	75.5	78.0
6"	60.1	62.0	63.9	62.6
6'-OCH_3	-	-	-	56.2
CO	-	-	168.0	-
1'''	-	-	122.7	-
2'''	-	-	110.1	-
3'''	-	-	145.7	-
4'''	-	-	135.7	-
5'''	-	-	145.7	-
6'''	-	-	110.1	-

EXPERIMENTAL

General. Melting points were determined using a Gallenkamp apparatus and are uncorrected. The UV-VIS spectra were determined using Pye-Unicam SP8-150 and PE Lambda-3 spectrophotometers while IR spectra were run as KBr pellets using PE 467 and Pye-Unicam SP3-300 instruments.

The ^1H and ^{13}C NMR spectra were obtained with a 400 MHz instrument at the University of Maryland, College Park, USA. in either DMSO-d_6 or MeOH-d_4 with TMS as internal standard. EI-MS at 70 eV were obtained at the same institution while the FAB-MS were done at H.E.J. Research Institute, Pakistan.

Plant material. The aerial parts of *P. senegalense* were collected from within Nairobi in July 1991 and November 1992 and identified at the Department of Botany Herbarium, University of Nairobi where voucher specimen are stored. The fresh plant material was washed with acetone to remove the surface exudate as described before [2]. The leaves were then dried, ground and extracted twice with 70% and 50% aqueous MeOH. The extracts were combined and the MeOH was removed under reduced pressure. The residue was then partitioned into CH_2Cl_2 and EtOAc; the former partitioning was to remove residual surface flavonoids while the latter retrieved the internal tissue flavonoids: aglycones (artefacts) and glycosides.

Isolation and characterization. 20 g of the EtOAc fraction was chromatographed on Merck silica gel (70-230 mesh) previously soaked in 3% oxalic acid solution, dried and packed under $CHCl_3$. Elution in a step gradient manner with MeOH-$CHCl_3$ (up to 10% MeOH in $CHCl_3$) gave quercetin, kaempferol and luteolin. Fractions eluted with 15% MeOH in $CHCl_3$ yielded compound **1**. Further elution with 20% MeOH-$CHCl_3$ yielded 0.7 g of a fraction with compound **2** which was further purified by passing through Sephadex LH-20 to give 30 mg of compound **2**. Continued elution with 20% MeOH in $CHCl_3$ gave 0.5 g of a fraction containing compound **4** which was further purified on Sephadex LH-20 ($CHCl_3$-MeOH 1:1 v/v) to give 76 mg of compound **4**. Fractions eluted with 50% MeOH in $CHCl_3$ gave 150 mg of compound **3**.

Quercetin-3-β-O-galactoside (1). M.p. 230-232° [lit [7] m.p. 230-233°]; R_f 0.32 on silica gel with $CHCl_3$-MeOH (4:1 v/v). UV λ_{max} (MeOH) nm: 359.5, 258.0 shifts with NaOMe, NaOAc, NaOAc-H_3BO_3, $AlCl_3$ and $AlCl_3$-HCl; MS *m/z* (rel. int.): 448 (3), 386 (13), 338 (8), 316 (8), 302 (100), 268 (81), 272 (86); FAB-MS mass peak at *m/z* 464; ^{1}H NMR (DMSO-d_6): see Table 1; ^{13}C NMR (DMSO-d_6): see Table 2. Hydrolysis of **1** with HCl resulted in quercetin (m.p. 313-314°) and galactose.

Quercetin-3-β-O-glucoside (2). M.p. 220-222° [lit [6] m.p. identical]; R_f 0.12 on silica gel with $CHCl_3$-MeOH (4:1 v/v); UV λ_{max} (MeOH) nm: 255.9, 360.1 shifts with NaOMe, NaOAc, NaOAc-H_3BO_3, $AlCl_3$, $AlCl_3$-HCl; IR (KBr) cm^{-1}: 3300 (OH), 1670 (C=O); FAB-MS *m/z* (%): 646 (6), 374 (7), 358 (29), 343 (9), 328 (49), 318 (26), 302 (100), 285 (27), 272 (17); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2. Acid hydrolysis of **2** gave quercetin and glucose.

2'-β-O-glucosyl-4'-hydroxy-6'-methoxydihydrochalcone (4). M.p. 191-193°; UV λ_{max} (MeOH) nm: 343 sh, 308, 276 shifts with NaOMe and NaOAc; IR (KBr) cm^{-1}: 3300 (O-H), 1680 (C=O); EI-MS *m/z* (rel. int.): 435 (0.2), 369 (0.2), 303 (0.4), 277 (100), 272 (19), 255 (5), 241 (100), 167 (100); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2. The DEPT spectrum at 0 = 90° showed negative peaks at δ 31.2, 47.5 and 62.6. Hydrolysis of **4** with 2 N HCl gave compounds identical in all respects with 2',4'-dihydroxy-6'-methoxydihydrochalcone and glucose.

Quercetin-3-(2"-galloylglucoside) (3). M.p. 204-205° [lit. [8] m.p. 203-205°]; UV λ_{max} (MeOH) nm: 359.9, 289.0, 259.2 shifts with NaOMe, NaOAc, NaOAc-H_3BO_3, $AlCl_3$ and $AlCl_3$-HCl; IR (KBr) cm^{-1}: 3300, 1660, 1620; FAB-MS *m/z*: 616; EI-MS *m/z* (rel. int.): 358 (7), 302 (100), 273 (12), 170 (5), 153 (42), 137 (24); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2. Hydrolysis of **3** with HCl gave quercetin, glucose and gallic acid (reaction with ferric chloride).

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REFERENCES

1. Part I: Midiwo, J.O.; Matasi, J.J.; Wanjau, O.M.; Mwangi, R.W.; Waterman, P.G., Wollenweber, E. *Bull. Chem. Soc. Ethiop.* **1990**, 4, 123.
2. Part II: Midiwo, J.O.; Gikonyo, N.J.; Wanjau, O.M.; Matasi, J.J.; Waterman, P.G., *Bull. Chem. Soc. Ethiop.* **1992**, 6, 119.
3. Midiwo, J.O.; Gikonyo, N.K.; Mwangi, R.W. *Ins. Sci. its Appl.* **1994**: in press.
4. Arya, R; Babu, V.; Ilyas, M.; Nasim, K.T. *Phytochemistry* **1992**, 31, 2569.
5. Markham, K.R. *Techniques of flavonoid identification*, Academic press: London; 1982.
6. Bennini, B.; Chulia, A.J.; Kaouadji, M.; Thomasson, B.F. *Phytochemistry* **1992**, 31, 2483.
7. Horhammer, L.; Volf, E. *Arch. Pharm.* **1955**, 58, 288.
8. Dossaji, S.F.; Kubo, I. *Phytochemistry* **1980**, 19, 482.
9. Hufford, C.D.; Oguntimein, B.O. *J. Nat. Prod.* **1982**, 45, 337.