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FLAVONOIDS OF *EQUISETUM* SPECIES

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Abstract—A survey of *Equisetum* species found in British Columbia showed that they all contained flavonoid di- and tri-glycosides especially those of kaempferol and, to a lesser extent, those of quercetin. Three other minor flavonoids were identified as gossypitrin, herbacitrin and apigenin-4′-glucoside.

INTRODUCTION

THE FLAVONOIDS of *Equisetum*, commonly known as horsetails, were first studied by Nakamura and Hukuti[1] who isolated quercetin-3-glucoside, kaempferol-7-diglucoside and luteolin-5-glucoside from *E. arvense*. Later *E. palustre* was shown to contain kaempferol-7-glucoside, -3-rutinoside-7-glucoside and a pentaglycoside containing glucose and rhamnose. Gossypitrin was isolated and identified in *E. telmateia*.[3] The occurrence of a number of species of *Equisetum* in British Columbia and the obvious gap in our knowledge of the flavonoid chemistry of this interesting group of plants prompted us to survey the local species.

RESULTS AND DISCUSSION

The flavonoids of *Equisetum* were extracted, fractionated, and identified according to the standard procedures. The results of the survey are outlined in Table 1, while the chemical methods are given in Table 2 and discussed below.

The Monoglycosides

Two kaempferol glycosides were isolated and identified as kaempferol-3-glucoside and kaempferol-7-glucoside. Both gave kaempferol and glucose on acid hydrolysis. The 7-glucoside was identified through its UV spectra (no shift with NaOAc) (see Table 3), R_fs and the fact that it gave no intermediates on mild hydrolysis. Two monoglucosides of quercetin, viz. quercetin-3-glucoside and quercetin-7-glucoside were isolated and shown to be identical with authentic samples.

Four other monoglucosides were isolated. Herbacitrin was found to be present in *E. fluviatile* and *E. sylvaticum*. Confirmation of identity was shown by its UV data (no shift with NaOAc), R_fs and NMR. A second herbacetin glucoside was found in *E. variegatum* but did not co-chromatograph with herbacitrin. It gave herbacetin and glucose on acid hydrolysis, while partial hydrolysis gave no intermediate. The UV data indicates that position 7 is free, while the $AlCl_3$ shift ($\Delta\lambda = 43$) is less than that of herbacitrin ($\Delta\lambda = 62$)

[1] H. NAKAMURA and G. HUKUTI, *J. Pharm. Soc. Japan*, **60**, 449 (1940).
[2] S. BECKMANN and H. GEIGER, *Phytochem.* **2**, 281 (1963).
[3] J. E. HALL, Ph.D. Thesis, Chemistry Dept., Univ. of British Columbia (1967).

TABLE 1. DISTRIBUTION OF FLAVONOID GLYCOSIDES IN *Equisetum* SPECIES

	Kaempferol								Quercetin						Apigenin
	-3-glucoside	-7-glucoside	-3-diglucoside	-3-rutinoside	-3,7-diglucoside	-3-diglucoside-7-glucoside	-3-glucoside-7-diglucoside	-3-rutinoside-7-glucoside	-3-glucoside	-7-glucoside	-3-diglucoside	-3,7-diglucoside	-3-diglucoside-7-glucoside	Gossypitrin / Herbacitrin	-4'-glucoside
E. arvense L.	+	+			+				+					+ +	+
E. fluviatile L. (*a*)		+			+										*a*
(*b*)	+	+			+	+				+					
E. hiemale L.					+	+	+								
E. laevigatum A. Br.		+	+		+	+					+	+	+		
E. palustre L.					+	+		+							
E. pratense Ehrh.			+	+	+	+		+							
E. scirpoides Michx.	+				+	+									
E. sylvaticum L.	+	+	+		+				+*b*		+		+	+	
E. telmateia Ehrh.	+	+		+	+			+							
E. variegatum Schleich.	+	+	+		+	+	+						+	*c*	

a—Contains apigenin only.
b—Also contains a quercetin-3-glucose of a different R_f.
c—Contains a different herbacetin glucoside (3-glucoside?).

and suggests that the 3-hydroxyl is blocked;[4] it is therefore formulated as the 3-glucoside. Gossypitrin was found only in *E. fluviatile* and its identity was confirmed with an authentic sample as well as by NMR (for comparison with herbacitrin).

TABLE 2. CHEMICAL INVESTIGATION OF THE KAEMPFEROL AND QUERCETIN DI- AND TRI-GLYCOSIDES*

	Acid hydrolysis	H_2O_2 oxidation	Partial† acid hydrolysis
Kaempferol			
3-diglucoside	Kaempferol, glucose	Diglucose‡	Kaempferol-3-glucoside
3-rutinoside	Kaempferol, glucose rhamnose	Rutinose§	Kaempferol-3-glucoside
3,7-diglucoside	Kaempferol, glucose	Glucose	Kaempferol-7-glucoside
3-diglucoside-7-glucoside	Kaempferol, glucose	Diglucose‡	Kaempferol-7-glucoside, kaempferol-3,7-diglucoside
3-rutinoside-7-glucoside	Kaempferol, glucose rhamnose	Rutinose§	Kaempferol-7-glucoside, kaempferol-3,7-diglucoside
3-glucoside-7-diglucoside	Kaempferol, glucose	Glucose	Kaempferol-7-glucoside, + kaempferol-7-diglucoside‖
Quercetin			
3-diglucoside	Quercetin, glucose	Diglucose‡	Quercetin-3-glucoside
3,7-diglucoside	Quercetin, glucose	Glucose	Quercetin-7-glucoside
3-diglucoside-7-glucoside	Quercetin, glucose	Diglucose‡	Quercetin-7-glucoside, + quercetin-3,7-diglucoside

* All compounds given below gave no acylated groups on alkaline hydrolysis.
† Only the intermediates are given.
‡ The diglucose does not co-chromatograph with gentiobiose (see discussion for *RG* values).
§ Co-chromatographs with rutinose from rutin.
‖ Identified through R_fs, UV and both total & partial acid hydrolysis.

[4] L. JURD, *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), MacMillan, New York (1962).

TABLE 3. UV SPECTRA OF FLAVONOID GLYCOSIDES ISOLATED FROM *Equisetum*

	Ethanol λ_{max} (nm)	△λ $AlCl_3$ (band II)	△λ NaOAc (band I)	△λ Boric acid (band II)	△λ NaOEt (band II)
Kaempferol					
7-glucoside	269,325*,368	59	0	—	Unstable
3-diglucoside	267·5,295*,350	45	10	—	Stable
3-rutinoside	267·5,295*,352	45	10	—	Stable
3,7-diglucoside	267,325*,352	47	0	—	Stable
3-diglucoside-7-glucoside	267·5,320*,350	43	0	—	Stable
3-glucoside 7-diglucoside	267,320*,350	42	0	—	Stable
3-rutinoside-7-glucoside	267·5,320*,350	44	0	—	Stable
Quercetin					
3-diglucoside	258,269*,297*,360	50	17	24	Stable
3-diglucoside-7-glucoside	258,270*,295*,361	51	2	27	Stable
Gossypitrin	263,280*,345,391	74	0	—	—
Herbacetin					
7-glucoside	279,337,388	62	0	—	—
3-glucoside(?)	273·5,327*,358	43	10	—	—
Apigenin					
4′-glucoside	270,325	55	8	—	Stable

* Inflection.

Finally, only one flavone glucoside was found in *E. fluviatile*. This was identified as apigenin-4′-glucoside. It had identical R_fs as the 7-glucoside and in fact co-chromatographed with it. It differed only in its colours in UV, while it gave a shift with NaOAc (free 7-hydroxyl group) as well as $AlCl_3$ (free 5-hydroxyl group). Its stability in the presence of NaOEt also confirms that the 7-hydroxyl group is free.

The Diglycosides

Three kaempferol diglycosides and two quercetin diglucosides were identified. The results of the chemical investigation are outlined in Table 2, and the only point that needs comment is the nature of the diglycosides present. Thus kaempferol and quercetin-3,7-diglucoside both gave only glucose on oxidation with H_2O_2, while the corresponding 7-glucosides were identified as intermediates during partial hydrolysis. In the case of kaempferol-3-rutinoside, H_2O_2 oxidation gave rutinose identified by chromatography with an authentic sample.

H_2O_2 oxidation of both kaempferol and quercetin-3-diglucoside gave a diglucoside which did not co-chromatograph with an authentic sample of gentiobiose. (R_G of the diglucoside: BAW = 0·47, BBPW = 0·55; R_G of gentiobiose: BAW = 0·30, BBPW = 0·40). The possibility is that the diglucoside is sophorose (β, 1→2 linkage), which is of more frequent occurrence than gentiobiose (β, 1→6 linkage). The R_fs are also in agreement with those recorded for sophorosides.[5]

[5] J. B. HARBORNE, *Comparative Biochemistry of Flavonoids*, Academic Press, London (1967).

TABLE 4. R_fS OF FLAVONOID GLYCOSIDES ISOLATED FROM *Equisetum*

Flavonoids	R_fs* (×100)				
	H_2O	HOAc	BAW	TBA	PhOH
Kaempferol					
3-glucoside	28	43	81	65	64
7-glucoside	3	12	61	40	46
3-diglucoside	44	59	58	40	49
3-rutinoside	43	55	64	48	53
3,7-diglucoside	55	68	36	17	51
3-diglucoside-7-glucoside	81	83	15	8	45
3-glucoside-7-diglucoside	81	88	4	1	29
3-rutinoside-7-glucoside	65	74	22	11	46
Quercetin					
3-glucoside	21	34	68	53	35
7-glucoside	2	7	32	20	19
3-diglucoside	49	62	45	32	33
3,7-diglucoside	43	55	21	10	—
3-diglucoside-7-glucoside	71	81	10	6	20
Gossypetin					
7-glucoside	2	7	23	15	11
Herbacetin					
7-glucoside	3	11	44	29	28
Apigenin					
4′-glucoside	9	23	71	60	72

* See Experimental for solvent key.

The Triglycosides

Three kaempferol and one quercetin triglycoside were identified. The chemical investigation of all four triglycosides is outlined in Table 2. Oxidation of kaempferol-3-rutinoside-7-glucoside with H_2O_2 gave rutinose. The diglucoside obtained by H_2O_2 oxidation of both kaempferol-3-diglucoside-7-glucoside and quercetin-3-diglucoside-7-glucoside was found to be identical with that obtained from oxidation of kaempferol- and quercetin-3-diglucosides. It is probably sophorose as it did not co-chromatograph with gentiobiose.

The corresponding 3,7-diglucosides as well as the 7-glucoside were identified as intermediates during partial acid hydrolysis.

In the case of kaempferol-3-glucoside-7-diglucoside, H_2O_2 oxidation gave only glucose, while partial acid hydrolysis gave the 7-diglucoside as well as the 7-monoglucoside as intermediates. The former (7-diglucoside) was identified through its R_fs, UV data, colour properties (yellow in UV) and acid hydrolysis which gave the 7-monoglucoside as an intermediate.

Other Glycosides

No flavonol pentaglycosides, as reported by Beckmann and Geiger,[2] were detected during this study. It is significant that the analytical data quoted[2] for the kaempferol pentaglycoside could equally well refer to the kaempferol 3-diglucoside-7-glucoside now reported. (Found: C, 47·3; H, 5·78, Pentaglycoside + 3 moles H_2O requires C, 47·6; H, 5·86. Triglucoside + 3 moles H_2O requires C, 47·9; H, 5·50%.) Furthermore, the possibility of breakdown of a pentaglycoside under the conditions of extraction and analysis is doubtful since all the other glycosides isolated were stable under a variety of conditions.

The luteolin 5-glucoside reported by Nakamura and Hukuti[1] in *E. arvense* also was not detected in this or any other *Equisetum* species. Harborne[6] was similarly unable to confirm the occurrence of this 5-glucoside in several species.

We can only conclude that the above reports[1,2] are in error.

EXPERIMENTAL

Plant material. The plants were collected in British Columbia in late summer and only barren stems were analysed; voucher specimens have been retained in the Botany Department, University of British Columbia.

Extraction. The plant material was extracted with 60% EtOH, concentrated under vacuum, freeze-dried and fractionated on a polyamide column prior to paper chromatography.

Hydrolytic procedures. Total acid hydrolysis was carried out with 2 N HCl, while partial acid hydrolysis was carried out with 0·05 N HCl over a period of 1 hr. Alkaline hydrolysis was carried out with N NaOH in N_2. The H_2O_2 oxidation was performed according to the procedure of Chandler and Harper.[7]

Chromatography. Standard methods were followed using Whatman No. 1 and No. 3 MM paper. The following solvents were employed: H_2O; 15% HOAc; BAW: *n*-BuOH–HOAc–H_2O (4:1:5); TBA: *t*-BuOH–HOAc–H_2O (3:1:1); PhOH–H_2O (4:1, w/v); BBPW: benzene–*n*-BuOH–pyridine–H_2O (1:5:3:3).

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[6] J. B. Harborne, *Phytochem.* **6,** 1569 (1967).
[7] B. V. Chandler and K. A. Harper, *Austral. J. Chem.* **14,** 586 (1961).

Key Word Index—Equisetum; Sphenopsida; flavonoids; chemotaxonomy; kaemperol-di-glycosides; quercetin.