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STYRYLPYRONE GLUCOSIDES FROM *EQUISETUM*

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Key Word Index—*Equisetum arvense*; *Equisetum* sp.; Equisetaceae; rhizomes; styrylpyrones; phenolics; NMR.

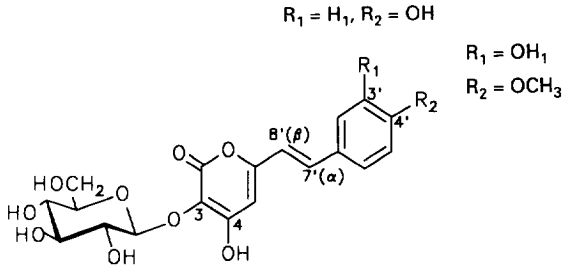
Abstract—Two styrylpyrone glucosides, 3′-deoxyequisetumpyrone (3,4-hydroxy-6-(4′-hydroxy-*E*-styryl)-2-pyron-3-*O*-β-D-glucopyranoside) and 4′-*O*-methylequisetumpyrone (3,4-hydroxy-6-(3′-hydroxy-4′-methoxy-*E*-styryl)-2-pyron-3-*O*-β-D-glucopyranoside) have been isolated from the rhizomes of *Equisetum arvense*. The structures of the new derivatives were elucidated spectroscopically. These compounds are accumulated as the main phenolics in rhizomes and gametophytes of all *Equisetum* species. In these organs, they represent a sink for compounds derived from hydroxycinnamoyl CoA esters and malonyl units, whereas sporophytic shoots contain no styrylpyrones but a considerable variety of flavonoid glycosides.

INTRODUCTION

We reported previously the isolation of equisetumpyrone (**1**) from gametophytes of *Equisetum arvense* [1]. While several styrylpyrones have been reported from higher plants [2–4] and fungi [5, 6], this was the first report of a glycosylated styrylpyrone. More recently, these compounds have been found to be characteristic phenolics in gametophytes and rhizomes of other *Equisetum* species as well [7]; in these organs, they generally replace the flavonoid glycosides. In green organs, during the course of generative reproduction, there is a switch from styrylpyrone biosynthesis in the haploid gametophytes to flavonoid biosynthesis in the diploid sporophytes [7]. Herein we report the isolation and structural elucidation of further styrylpyrone glucosides which are present in methanolic extracts of rhizomes and gametophytes of *Equisetum* species.

RESULTS AND DISCUSSION

Compounds **2** and **3** were isolated together with **1** and caffeic acid conjugates from a methanolic extract of lyophilized rhizomes of *E. arvense*. Purification was achieved by repeated CC on polyamide, Sephadex LH-20 and Sephadex G-25-80 and reverse phase HPLC. The negative-ion FAB-mass spectrum gave $[M - H]^-$ at *m/z* 407 for **2** and at *m/z* 437 for **3**. The structures of **2** and **3** were established by 1D and 2D ^1H and ^{13}C NMR



experiments. The presence of a β-linked glucose and an *E*-styrylpyrone moiety was evident from the characteristic shifts and couplings in these spectra (Table 1) and by comparison with data on styrylpyrones published earlier [1, 8]. Further evidence was obtained from the UV spectra of **1** and **2** which show absorbance bands typical of styrylpyrones [1]. The site of attachment of the glucose moiety was confirmed by 2D ^{13}C–H COSY, optimized for long-range couplings, and the attachment at the 3-OH was established from the correlation between H-1″ and C-3 in both compounds. Compatible with this interpretation was the absence of any nuclear overhauser enhancements to either H-5 or the styryl systems in the 2D NOESY spectra. The substitution patterns of the styryl moieties were evident from the shifts and characteristic couplings in the ^1H NMR spectra (Table 1). The site of methylation in **3** was confirmed by a NOE interaction of the methyl protons with H-5′ in the 2D NOESY spectrum. Thus, the structures of **2** and **3** were clearly defined as 3,4-hydroxy-6-(4′-hydroxy-*E*-styryl)-2-pyron-3-*O*-β-D-glucopyranoside (**2**) and 3,4-hydroxy-6-(3′-hydroxy-4′-methoxy-*E*-styryl)-2-pyron-3-*O*-β-D-gluco-

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Table 1. ^{13}C and ^{1}H NMR spectral data for compounds **1**–**3** in DMSO-d_6 at 300 MHz

	1 ([1])		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	160.5 *s*		162.2 *s*		162.2 *s*	
3	127.7 *s*		124.0 *s*		124.0 *s*	
4	162.4 *s*		168.7 *s*		168.7 *s*	
5	103.5 *d*	6.10 *s*	107.6 *d*	5.89 *s*	108.0 *d*	5.93 *s*
6	154.6 *s*		154.1 *s*		153.9 *s*	
1′	127.1 *s*		126.8 *s*		128.8 *s*	
2′	113.9 *d*		128.7 *d*	7.41 *d* (8.3)	113.4 *d*	7.00 *d* (2.0)
3′	145.5 *s*		115.8 *d*	6.76 *d* (8.1)	146.7 *s*	
4′	146.9 *s*		158.3 *s*		148.6 *s*	
5′	115.7 *d*	6.74 *d* (8.1)	115.8 *d*	6.76 *d* (8.1)	112.2 *d*	6.90 *d* (8.6)
6′	119.9 *d*	6.90 *dd* (8.3, 2.1)	128.7 *d*	7.41 *d* (8.3)	119.5 *d*	6.99 *dd* (2.0, 8.6)
7′ (α)	132.9 *d*	6.98 *d* (16.3)	131.9 *d*	6.94 *d* (16.0)	131.4 *d*	6.94 *d* (16.0)
8′ (β)	116.4 *d*	6.60 *d* (16.0)	117.4 *d*	6.61 *d* (16.0)	118.3 *d*	6.58 *d* (16.0)
1″	104.2 *d*	4.67 *d* (6.7)	107.1 *d*	4.38 *d* (6.3)	107.1 *d*	4.38 *d* (6.3)
2″	73.7 *d*	3.19–3.18 *m*	73.7 *d*	3.18–3.09 *m*	73.7 *d*	3.18–3.09 *m*
3″	76.4 *d*	3.19–3.14 *m*	76.4 *d*	3.50–3.46 *m*	76.4 *d*	3.50–3.46 *m*
4″	69.6 *d*	3.14–3.07 *m*	69.9 *d*	3.74–3.71 *m*	69.9 *d*	3.74–3.71 *m*
5″	77.2 *d*	3.14–3.07 *m*	77.0 *d*	3.18–3.09 *m*	77.0 *d*	3.17–3.09 *m*
6″	60.8 *t*	A3.64 *d* (11.5, 1.7)	61.0 *t*	3.74–3.71 *m*	61.0 *t*	3.74–3.71 *m*
		B3.54 *d* (11.7, 5.3)				
OME					55.7 *q*	3.77 *s*

Multiplicities by DEPT pulse sequence. Data for **1** obtained from samples stored and kept cool in DMSO-d_6 over a period of three months. Data from **2** and **3** obtained from freshly prepared samples; for details see text.

pyranoside (**3**), respectively. We found no broadening of NMR signals for **2** and **3** as described previously for **1** [1]. This may be explained by different exhange rates in DMSO, which depend a lot on the amount of water present in the sample. Data from different measurements of **1** also suggest that the shift of H-5 correlates with the amount of water present in the sample. Thus, in freshly prepared samples we observed chemical shifts at δ5.95, whereas in samples that had stood for some time or had been kept cool, a shift of 6.7 ppm was observed for the H-5 signal, due to the increased water content. The phenomenon was observed also for H-1″ signal with shifts of δ4.46 in freshly prepared samples which show a downfield shift to δ4.67 after three months of storage. The opposite was true for the C-2, C-4, C-5 and C-1″ signals which were shifted upfield, C-4 considerably from δ168.1 initially to δ162.4 after three months of storage. Such phenomena may account for differences between the data published for **1** and the data for **2** and **3** presented here (Table 1).

EXPERIMENTAL

Isolation and purification. Freeze-dried and powdered rhizomes from *E. arvense* L. were extracted several times with MeOH and MeOH–NH_4OH at room temp. Compounds **2** and **3** were isolated from the combined and concd extracts by polyamide CC. Initially the column was washed with MeOH and then **2** and **3** were eluted together with other acidic compounds using MeOH–NH_4OH (97:3). Further purification was achieved by CC on RP-18 (Merck) using a MeOH–H_2O gradient and Sephadex LH-20 (Pharmacia) using MeOH–H_2O (1:19). Final purification was achieved by CC on Sephadex G25–80 (Pharmacia) with water.

HPLC analysis. Conditions used were as reported previously [1]. R_ts and maxima of the on-line UV spectra (most intense band underlined): **1**, 19.8 min; 220, 253, 372 nm; **2**, 26.2 min, 213 (sh), 270, 367 nm; **3**, 29.7 min, 218, 252, 370 nm (int. standard: quercetin-3-*O*-glucoside, R_t = 30.2 min). The overall levels of styrylpyrone glucosides in the rhizomes were in the range of 0.4 to 1.2% dry wt, whereas in rhizome tips the levels were up to 4.2% dry wt. In axenic cultivated gametophytes, the accumulated amount increased during subculture from *ca* 0.1% dry wt initially, to 0.9% dry wt after 6 weeks.

TLC analysis. TLC was performed on silica HPTLC plates, 0.1 mm (Kieselgel 80, Merck,) solvent: EtOAc–H_2O–HOAc–HCO_2H (50:13:6:6). Detection: UV 366 nm. Spot appearance: fluorescent green-blue. Spray reagent: diphenyl-boric acid ethanolamine complex (NA) 1% in MeOH; spot appearance: fluorescent yellow.

UV. Maximum (nm) in MeOH: **2**, 224, 264, 300sh, 309, 328h, 360; **3**, 226, 247 sh, 252, 307sh, 361.

NMR and MS. All 1D and 2D ^{1}H and ^{13}C NMR spectra were recorded at 303 K in DMSO-d_6 with a standard 5 mm dual frequency probe. The 2D ^{1}H–^{13}C long-range COSY was collected under inverse conditions with the Brucker-supplied INVDR2LP pulse sequence optimized for ~ 8 Hz long-range couplings. FAB-MS were measured using Xe as bombarding gas and glycerol as matrix. Operations were carried out at ambient temp.

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