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EFFUSIDES I–V: 9,10-DIHYDROPHENANTHRENE GLUCOSIDES FROM *JUNCUS EFFUSUS*

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Key Word Index—*Juncus effusus*; Juncaceae; 9,10-dihydrophenanthrene glucosides; effusides.

Abstract—Five 9,10-dihydrophenanthrene glucosides, named effusides I–V, have been isolated from the methanolic extract of *Juncus effusus*. Structures have been determined on spectroscopic grounds.

INTRODUCTION

In a chemical investigation of *Juncus effusus*, connected to a study of the allelopathic interactions between freshwater macrophytes and microalgae [1], we have recently reported the isolation of some 9,10-dihydrophenanthrene derivatives [2–4]. In pursuing such a study we now describe the isolation of five glucosides named effusides I–V.

Effusides I and V were identified as the 12-*O*-β-D-glucopyranoside (**1**) and the 2,12-di-*O*-β-D-glucopyranoside (**5**) of 1,8-dimethyl-2-hydroxy-5-hydroxymethyl-7-methoxy-9,10-dihydrophenanthrene (**6**), while effusides II–IV were attributed structures 7-*O*-(**2**), 2-*O*-(**3**) and 12-*O*-β-D-glucopyranosyl-1,8-dimethyl-2,7-dihydroxy-5-hydroxymethyl-9,10-dihydrophenanthrene (**4**).

The less polar effuside I (**1**) had a molecular formula, $C_{24}H_{30}O_8$, according to the presence of 24-carbon signals in the ^{13}C NMR spectrum (Table 1) and a quasimolecular ion at *m/z* 469 in the FAB mass spectrum. The ^1H NMR spectrum (Table 2) showed two aromatic *ortho* coupled doublets at δ 6.76 and 7.38, an aromatic singlet at δ 7.14, two AB doublets at δ 4.68 and 4.91, a methoxyl methyl at δ 3.85, four benzylic protons as a multiplet at δ 2.62 and two methyl singlets at δ 2.17 and 2.18, beside an anomeric proton at δ 4.32 and further signals of a saccharide moiety. The signal at δ 6.76, attributed to the H-3 proton and correlated to the carbon at δ 111.9 in the H–C one-bond COSY, gave cross peaks in the H–C long-range COSY with the signals at δ 121.4 and 128.5 which were attributed to the C-1 and C-4a carbons. Accordingly, both these carbons were correlated to the H-10 benzylic protons at δ 2.62 while the C-1 gave an additional cross peak with the H-11 methyl protons at δ 2.18. The signal at δ 7.38, linked to the carbon at δ 126.4 and attributed to the H-4 proton, was correlated to the OH-bearing C-2 carbon at δ 154.0, and to the signals at δ 137.9 and 125.0. These signals, also correlated to the benzylic signal at δ 2.62, were attributed to the C-1a and C-5a carbons, respectively, owing to the heterocorrelations of the latter with the H-12 protons at δ 4.68 and 4.91 and with the H-6 proton at δ 7.14, linked to the carbon at δ 111.0. This latter proton, together with the methyl protons at δ 2.17, gave cross peaks with the C-8 carbon at δ 119.8. The chemical shifts of the C-6 and C-8 carbons agreed with the presence of the methoxyl group at C-7 and in a NOESY spectrum the methyl of this group at δ 3.85 gave nOe interaction with the H-6 proton.

Enzymatic hydrolysis of **1** gave D-glucose, identified by GC analysis [5], and aglycone **6**. The coupling of the H-1 proton of glucose agreed with a β-configuration at the anomeric carbon and the differences in chemical shift and multiplicity of the H-12 protons in **1** and **6** justified the location of the saccharide residue at the C-12 position. The nOe interactions in a NOESY experiment of the anomeric proton with the H-12 methylene confirmed the structure.

Effuside II (**2**) had 23 carbon signals in the ^{13}C NMR spectrum and a quasimolecular peak at *m/z* 455 in the FAB mass spectrum for the molecular formula $C_{23}H_{28}O_8$. Enzymatic hydrolysis gave aglycone **7** and D-glucose, and the coupling of the anomeric proton indicated a β configuration of the sugar. The NMR spectra of **2** were lacking in the methoxyl methyl signals and a comparison with those of **1** evidenced a significant upfield shift of the H-6 proton and a downfield shift of the C-6 carbon. These data agreed well with the presence of a hydroxyl group rather than a methoxyl one at C-7.

Effuside III (**3**) had the same molecular formula $C_{23}H_{28}O_8$ of **2** and by enzymatic hydrolysis gave **7** and D-glucose. It showed in the ^1H NMR spectrum the H-12 protons as a sharp singlet shifted upfield at δ 4.48 and the anomeric proton shifted downfield at δ 4.82. These elements suggested that the saccharide moiety was linked to

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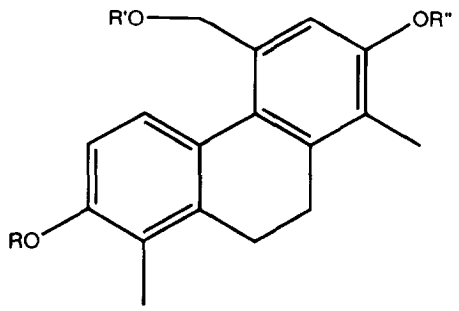

1 R = H R' = Glc R" = Me
2 R = H R' = Glc R" = H
3 R = H R' = H R" = Glc
4 R = Glc R' = H R" = H
5 R = Glc R' = Glc' R" = Me
6 R = H R' = H R" = Me
7 R = H R' = H R" = H

Table 1. ^{13}C NMR chemical shifts of effusides I–V

C	1	2	3	4	5
1	121.4	120.9	122.5	123.0	123.1
2	154.0	153.7	153.9	154.0	154.3
3	111.9	111.9	111.7	111.9	112.0
4	126.4	126.2	126.3	125.9	126.5
4a	128.5	128.0	129.1	128.5	127.8
1a	137.9	138.2	137.7	138.2	138.3
5	130.9	131.1	135.7	135.8	131.6
6	111.0	115.5	115.1	114.3	111.0
7	155.0	156.2	153.6	153.5	155.4
8	119.8	120.2	120.0	120.8	121.5
8a	138.3	138.2	138.5	138.0	138.3
5a	125.0	127.1	125.4	126.9	127.9
9	25.0	24.9	25.0	25.1	25.0
10	25.9	26.0	26.0	25.9	25.9
11	11.5	11.6	11.6	11.5	11.8
12	68.9	69.4	62.2	62.0	68.7
Me	11.5	11.6	11.8	11.8	11.5
OMe	55.4				55.5
Glc-1	101.6	101.1	101.6	101.3	101.6
2	73.5	73.5	73.4	73.4	73.5
3	76.8	76.8	77.0	77.0	76.9
4	70.1	70.1	69.7	69.8	69.7
5	76.6	76.8	76.7	76.6	76.6
6	61.1	61.1	60.8	60.8	60.7
Glc-1′					101.2
2′					73.4
3′					76.9
4′					70.0
5′					76.6
6′					61.0

an aromatic hydroxyl group and the nOe interaction between the anomeric proton and the aromatic singlet at δ 7.18 indicated the C-7 position.

Also effuside IV (**4**) had the same molecular formula and afforded by hydrolysis **7** and D-glucose. The H-3 proton was shifted downfield at δ 6.97 and gave nOe interaction with the anomeric proton according to the presence of the saccharide moiety at C-2.

Effuside V (**5**), the most polar compound, had a molecular formula $C_{30}H_{40}O_{13}$, on the basis of the FAB-MS and ^{13}C NMR data, and gave by hydrolysis **6** and D-glucose. It showed in the ^{1}H NMR spectrum the H-12 protons as two AB doublets at δ 4.70 and 4.88, the H-3 doublet at δ 7.00 and the H-6 singlet at δ 7.16. The comparison of these chemical shifts with those of the other effusides suggested that D-glucose was at C-2 and C-12 while the methoxyl group was at C-7. Accordingly in the NOESY experiment the anomeric proton at δ 4.29 was correlated to the H-12 protons, the anomeric proton at δ 4.84 gave interaction with the H-3 proton and the methyl at δ 3.82 was correlated to the H-6 proton.

EXPERIMENTAL

NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on a Bruker AC 400 spectrometer in DMSO-d_6 (aglycones in acetone-d_6). One bond and long-range H–C COSY experiments were performed with the XHCORR microprogramme using delays corresponding to $J_{C,H}$160 Hz and 8 Hz, respectively. EI mass spectra were obtained with a Kratos MS 50 apparatus and FAB mass spectra with a VG ZAB 2SE apparatus. DCCC was run with a mixture $CHCl_3$–MeOH–H_2O (13:7:4 for **1–4**, 26:14:5 for **5**) using the more polar upper layer as the mobile phase. Reverse-phase HPLC was performed using LiChrosorb RP8 column (MeOH–H_2O, 1:1) for **1–4** and LiChrosorb NH_2 (CH_3CN–H_2O, 4:1) for **5**.

Isolation of effusides. Juncus effusus, collected in the summer near Naples was air dried and extracted with Et_2O and then with MeOH. The MeOH extract (350 g) after removal of the solvent was distributed between EtoAc and H_2O.

The organic layer was chromatographed on silica gel and the fractions eluted with $CHCl_3$–MeOH (9:1) were rechromatographed on Sephadex LH-20 eluting with MeOH–H_2O (3:1). DCCC chromatography and HPLC chromatography of fractions 130–143 gave pure **1** (10 mg), **2** (8 mg), **3** (11 mg) and **4** (6 mg). The aq. layer was chromatographed on Amberlite and the MeOH fraction was distributed between *n*-BuOH and H_2O. DCCC and HPLC processes on the aq. layer gave pure **5** (11 mg).

Enzymatic hydrolysis of glucosides. Pure effuside (3 mg) in H_2O (0.5 ml) was treated with β-glucosidase (1 mg, Sigma) at 37° for 12 hr. The reaction mixture was extracted with EtOAc: the organic layer gave aglycone **6** (**7**) while HPLC chromatography (CH_3CN–H_2O, 4:1) of the

Table 2. ^{1}H NMR chemical shifts of effusides I–V

H	1	2	3	4	5
3	6.76 *d*	6.68 *d*	6.71 *d*	6.97 *d*	7.00 *d*
	(8.4)	(8.3)	(8.3)	(8.8)	(8.7)
4	7.38 *d*	7.31 *d*	7.33 *d*	7.37 *d*	7.43 *d*
	(8.4)	(8.3)	(8.3)	(8.8)	(8.7)
6	7.14 *s*	6.88 *s*	7.18 *s*	6.94 *s*	7.16 *s*
9	2.62 *m*	2.62 *m*	2.62 *m*	2.62 *m*	2.54 *m*
					2.63 *m*
10	2.62 *m*	2.62 *m*	2.62 *m*	2.62 *m*	2.54 *m*
					2.63 *m*
11	2.18 *s*	2.14 *s*	2.19 *s*	2.21 *s*	2.23 *s*
12	4.68 *d*	4.45 *d*	4.48 *s*	4.47 *s*	4.70 *d*
	(11.0)	(10.7)			(11.0)
	4.91 *d*	4.80 *d*			4.88 *d*
	(11.0)	(10.7)			(11.0)
Me	2.17 *s*	2.12 *s*	2.13 *s*	2.11 *s*	2.15 *s*
OMe	3.85 *s*				3.82 *s*
Glc-1	4.32 *d*	4.30 *d*	4.82 *d*	4.79 *d*	4.29 *d*
	(7.7)	(7.8)	(7.3)	(7.3)	(7.6)
2	3.12*	3.11*	3.26*	3.28*	3.05*
3	3.19*	3.20*	3.25*	3.25*	3.19*
4	3.18*	3.18*	3.10*	3.12*	3.15*
5	3.20*	3.20*	3.16*	3.15*	3.28*
6	3.52 *dd*	3.51 *dd*	3.46 *dd*	3.44 *dd*	3.45*
	(5.7 11.3)	(5.6 11.2)	(5.5 11.2)	(5.6 11.1)	
	3.63 *dd*	3.71 *dd*	3.69 *dd*	3.71 *dd*	3.69*
	(1.6 11.3)	(1.7 11.2)	(1.5 11.2)	(1.5 11.1)	
Glc-1′					4.84 *d*
					(7.1)
2′					3.28*
3′					3.26*
4′					3.08*
5′					3.18*
6′					3.45*
					3.69*

*Overlapping signals.

aq. layer gave D-glucose. The D-configuration was determined by treatment with L-cysteine methylester hydrochloride and TMS-imidazole [5].

Aglycone **6**. ^{1}H NMR δ 6.75 (*d*, 1H, J = 8.8 Hz, H-3), 7.43 (*d*, 1H, J = 8.8 Hz, H-4), 7.07 (*s*, 1H, H-6), 2.64 (*s*, 4H, H-9 and H-10), 2.21 (*s*, 3H, H-11), 4.69 (*s*, 2H, H-12), 3.83 (*s*, 3H, OMe), 2.16 (*s*, 3H, Me). Aglycone **7**: ^{1}H NMR δ 6.75 (*d*, 1H, J = 8.7 Hz, H-3), 7.39 (*d*, 1H, J = 8.7 Hz, H-4), 6.87 (*s*, 1H, H-6), 2.62 (*s*, 4H, H-9 and H-10), 2.18 (*s*, 3H, H-11), 4.52 (*s*, 2H, H-12), 2.15 (*s*, 3H, Me).

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