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ELLAGIC ACID DERIVATIVES AND NAPHTHOQUINONES OF *DIONAEA MUSCIPULA* FROM *IN VITRO* CULTURES*

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Key Word Index—*Dionaea muscipula*; Droseraceae; 1,4-naphthoquinones; ellagic acid methyl ethers and glucosides; 3,3′-di-*O*-methylellagic acid 4,4′-di-*O*-glucoside.

Abstract—From *Dionaea muscipula*, obtained by *in vitro* culture, the known compounds plumbagin, chloroplumbagin and 8,8′-biplumbagin as naphthoquinones, 1-*O*-β-galloylglucose, ellagic acid, 3-*O*-methylellagic acid, 3,3′-di-*O*-methylellagic acid and its 4-*O*-glucoside, and a new compound, the 4,4′-di-*O*-glucoside of 3,3′-di-*O*-methylellagic acid, were isolated. The assignments of NMR resonances of 3,3′-di-*O*-methylellagic acid 4-*O*-glucoside were substantiated by correlation techniques.

INTRODUCTION

Dionaea muscipula Ellis is a carnivorous plant [1] considered to be a source of an anticancer drug [2, 3]. The species has very specific environmental requirements [1]; hence, its supply from natural habitats is restricted. This difficulty, as in the case of other Droseraceae species, can be overcome by propagation by *in vitro* cultures [4, 5]. Previous investigation of the ground-grown plants showed the presence of the naphthoquinones plumbagin, chloroplumbagin, droserone and hydroplumbagin 4-*O*-glucoside [6]. In the present study, with the material obtained by *in vitro* culture, the naphthoquinones: plumbagin (**1**), chloroplumbagin (**2**), 8,8′-biplumbagin (3) and ellagic acid (**4**) and its derivatives: 3-*O*- and 3,3′-di-*O*-methyl ether (**5**, **6**), 3,3′-di-methylellagic acid 4-*O*-β-glucoside (**7**) and its 4,4′-di-*O*-β-glucoside (**8**) were isolated.

RESULTS AND DISCUSSION

The methanolic extract of the fresh whole plants was separated into a distillate of residual water, chloroform and water fraction, respectively. The distillate yielded compound **1**, the chloroform fraction compounds **1**–**3**, and the water fraction compounds **4**–**9**. Compounds **1** and **2** where identified as plumbagin and 3-chloroplumbagin, respectively, from their spectral (EIMS, UV, ^{1}H and ^{13}C NMR) [6–11] and co-TLC data. Compound **3** was 8,8′-biplumbagin (maritinone) on the basis of EIMS ($[M]^+$ at *m/z* 374) and ^{1}H NMR showing a symmetrical dimer and the expected absence of H-8 signal at *ca* δ_H 7.6 [8, 11]. Compound **9** was 1-*O*-β-galloylglucose according to UV, ^{1}H and ^{13}C NMR data [12]. Compounds **4**–**6** appeared to be ellagic, 3-*O*-methylellagic and 3,3′-di-*O*-methyl ellagic acids, respectively from their UV, EIMS, ^{1}H and ^{13}C NMR spectra compared with those previously reported [13–20] (Table 1).

Compound **7** gave ^{1}H and ^{13}C NMR spectra similar to those published for 4-*O*-β-glucoside of 3,3′-di-*O*-methylellagic acid [15]. The NOE between H-5 (δ_H 7.81) and anomeric H-1″ of glucosyl (δ_H 5.16) fixed the glucosidic linkage at C-4 position. The protonated carbons were determined from one-bond HC-COSY and the detailed assignment of ^{13}C resonances followed from ^{13}C/^{1}H NMR spectra before and after selective decouplings from particular protons of an aglycone and H-1″ of glucose (Table 1). The irradiation of methoxyl groups was not selective owing to their close shifts ($\Delta\delta = 0.04$) and resulted in simultaneous decoupling from both. The missing information about exact NMR shifts for the methoxyl from each position (C-3 or C-3′) could not be obtained from NOESY spectrum, as no correlation of glucose H-1″ and/or 4′-OH with any of methoxyls was observed. Finally, this asignment was obtained from HMBC correlation through three bonds (^3J) of C-3, δ_C 141.7 (also ^3J-correlated with H-5, δ_H 7.81) and C-3′, δ_C 140.1 (^3J-correlated with H-5′, δ_H 7.51) with methoxyls at δ_H 4.09 (OMe-3) and 4.05 (OMe-3′), respectively we could not assign the NMR resonances for **5** and **6** in the same manner owing to solubility problems, which have also been

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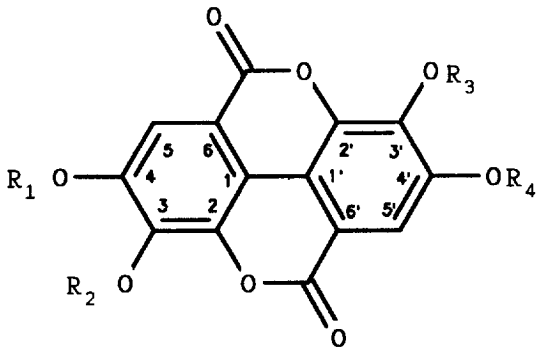
Table 1. ^{13}C NMR data for **4–8** (in DMSO-d_6)

C	6	8	C	7	
1 (1′)	111.6	115.2	1	114.0	*dd* (7.6/1.0) (H-5,5′)*
2 (2′)	141.1	142.1	2	140.8	*d* (1.0) (H-5)
3 (3′)	140.0	139.7	3	141.7	*dq* (7.5/4.0) (H-5, OMe-3)
4 (4′)	152.0	152.1	4	151.4	*dd* (3.0/3.0) (H-5, H-1″)
5 (5′)	111.3	111.0	5	111.8	*d* (167.7) (H-5)
6 (6′)	112.0	113.5	6	111.8	*d* (2.0) (H-5)
7 (7′)	158.3	158.8	7	158.2	*d* (4.5) (H-5)
MeO-3 (3′)	61.0	60.9	1′	111.0	*dd* (7.6/1.0) (H-5′/H-5)
1″ (1‴)		104.0	2′	141.5	*d* (1.5) (H-5′)
2″ (2‴)		73.4	3′	140.1	*ddq* (7.5/2.5/4) (H-5′, OH-4′, OMe-3′)
3″ (3‴)		76.2	4′	152.7	*t* (2.0/2.0) (H-5′, OH-4′)
4″ (4‴)		69.6	5′	111.5	*dd* (165.7/2.5) (H-5′, OH-4′)
5″ (5‴)		77.3	6′	112.7	*d* (1.5) (H-5′)
6″ (6‴)		60.8	7′	158.3	*d* (4.5) (H-5′)
			MeO-3	61.5	*q* (146.5) (MeO-3)
			MeO-3′	60.9	*q* (146.5) (MeO-3′)
			1″	101.2	*dm* (161.2) (H-1″)
			2″	73.2	*dm* (143.0) (H-2″)
			3″	76.4	*dm* (140.5) (H-3″)
			4″	69.4	*dm* (144.0) (H-4″)
			5″	77.2	*dm* (141.5) (H-5″)
			6″	60.5	*dm* (138.7) (H-6″)

*Multiplicities, coupling constants and coupled protons, respectively, obtained from fully coupled spectra before and after selective decouplings.



	R_1	R_2	R_3	R_4
4	H	H	H	H
5	H	Me	H	H
6	H	Me	Me	H
7	Glc	Me	Me	H
8	Glc	Me	Me	Glc

encountered by other authors [20]; they measured ^{13}CNMR spectra in DMSO-d_6 for 3,3′-di-*O*-methylellagic acid at elevated temperature to obtain a more concentrated solution, sufficient to obtain signal assignment by HMBC technique—however, some shifts reported differ markedly from those recorded at ambient temperature [15, 16, 19].

Compound **8**, had a UV spectrum similar to that of **6** and **7**. The ^1HNMR spectrum exhibited signals of aromatic ring proton(s) and methoxyl(s) at δ_H 7.54 (*s*), and 4.04 (*s*), respectively, as in **6**, and also that of an anomeric proton of sugar residue at δ_H 4.89 (*d*, J = 7.2 Hz), with intensities in a 1:3:1 ratio, respectively. Assuming **8** to be an ellagic acid derivative, such an

arrangement of signals requires a symmetrical molecule with two methoxyls and two glycosyl moieties. This assumption was in accord with ^{13}C NMR spectra (Table 1) which showed a set of seven signals indicative of an ellagic acid skeleton very similar to those of **6**, and also six signals indicative of glucopyranosyls and one for methoxyl groups. The δ_C 60.9 shift value of the latter indicated their attachment at C-3,3′ positions and hence glucosyls are placed at C-4,4′ positions (methoxyls at C-4,4′ would resonate at *ca* δ_C 56 [16]). Thus, **8** is 4,4′-di-*O*-*β*-glucoside of 3,3′-di-*O*-methylellagic acid, a new naturally occurring product.

Maritinone (**3**), ellagic acid (**4**) and its derivatives (**5**–**8**) and 1-*O*-galloylglucose (**9**) are new for the species and, except ellagic acid itself [21], also the family Droseraceae. Ellagic acid, a useful taxonomic marker [22], is considered a primitive character of several large systematic groups of the Dicotyledones, including subclass Dilleniidae to which the Droseraceae belongs [23]; it is considered to be an inhibitor of mutagenesis and carcinogenesis [25 and refs cited therein]. *O*-Methylated ellagic acid derivatives are considered to have a much more restricted occurrence [24].

EXPERIMENTAL

Plant material. Fully developed plants of *D. muscipula* obtained by *in vitro* culture on Reinerth–Mohr medium [4, 5] at the Botanic Garden, University of Wrocław, Poland were collected in 1992 (batch A) and 1993 (batch B).

Extraction and isolation. The whole fresh plants (separately from batches A, 392 g, and B, 339 g), were treated shortly with hot MeOH and extracted (3 ×) at room temp. The extract was concd at 40°, collecting separately residual water, from which **1** was obtained by $CHCl_3$ extraction. Dry extract was partitioned between $CHCl_3$ and H_2O. Fractions obtained from A and B had identical composition of phenolics according to TLC on silica gel (Merck) in toluene–HCO_2H (99:1) [6] and 2-D TLC on cellulose (Merck) in *n*-BuOH–H_2O–HOAc (4:1:5) and 15% HOAc. $CHCl_3$ fr. (B) yielded **1**–**3** after prep. TLC on silica gel (Merck) in toleuene–HCO_2H (99:1) and toluene (run × 1 or × 4). The H_2O fr. (A) was sepd by Sephadex-LH20 (Pharmacia) CC with H_2O → MeOH gradient into 15 frs Fr. 7 yielded **8** by prep. TLC on Avicel (Merck) in 15% HOAc and on polyamide-6 (Macherey-Nagel) in H_2O–EtOH–Ac_2CH_2 (4:2:1), respectively. Frs 8/9 gave **7** and **9**, resolved by prep. TLC on polyamide in $CHCl_3$–MeOH–MeCOEt–Ac_2CH_2 (9:4:2:1), each purified by TLC on Avicel in *n*-BuOH–H_2O–HOAc (4:1:5). Frs 14/18 yielded **4**, **5**, **6** after prep. TLC on Avicel in 50% HOAc. All compounds were cleaned by CC on Sephadex-LH 20 in MeOH, followed by crystallization. Yields: **1** (238 mg), **2** (38.3 mg), **3** (1 mg), **4** (3.1 mg), **5** (53.5 mg), **6** (7.5 mg), **7** (195 mg), **8** (7.3 mg), **9** (38 mg).

Spectroscopy. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR with TMS as int. standard.

3,3′-*Di*-O-*methylellagic acid* (**6**). UV λ_{max}^{MeOH}: 247, 261sh, 286sh, 360sh, 375, 414sh. EIMS *m/z* (rel. int.): 330 $[M]^+$ (100). ^{1}H NMR (DMSO-d_6): δ 7.51 (2H, *s*, H-5, H-5′), 4.04 (6H, *s*, 3-OCH_3, 3′-OCH_3). ^{13}C NMR: Table 1.

3,3′-*Di*-O-*methylellagic acid* 4-O-β-*glucoside* (**7**): UV λ_{max}^{MeOH}: 247, 261sh, 287sh, 353sh, 369, 406sh. EIMS *m/z* (rel. int.): 330 $[M - Glc + H]^+$ (100). LSI-MS *m/z* (rel. int.): 515 $[M + Na]^+$ (21), 493 $[M + H]^+$ (22), 492 $[M]^+$ (7), 330 $[M - Glc + H]^+$ (100). ^{1}H NMR (DMSO-d_6): δ 10.86 (1H, *s*, 4′-OH), 7.81 (1H, *s*, H-5), 7.50 (1H, *s*, H-5′), 5.16 (1H, *d*, J = 7.8 Hz, H-1″ of glucose), 4.09 (3H, *s*, 3-OCH_3), 4.05 (3H, *s*, 3′-OCH_3). ^{13}C NMR: Table 1.

3,3′-*di*-O-*methylellagic acid* 4,4′-*di*-O-β-*glucoside* (**8**). EI-MS: 330 $[M - 2 \times Glc + 2H]^+$ (100), 315 $[M - 2 \times Glc - Me]^+$ (65). LSI-MS *m/z* (rel. int.): 677 $[M + Na]^+$ (2), 654 $[M]^+$ (4) ($C_{28}H_{30}O_{18}$ req. 654), 330 $[M - 2 \times Glc + 2H]^+$ (100). UV λ_{max}^{MeOH}: 246sh, 253, 270sh, 310, 350. ^{1}H NMR (DMSO-d_6): δ 7.54 (2H, *s*, H-5, H-5′), 4.89 (2H, *d*, J = 7.2 Hz, H-1″ of glucose × 2), 4.04 (6H, *s*, 3-OCH_3, 3′-OCH_3). ^{13}C NMR NMR: Table 1.

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