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NAPHTHOQUINONES OF *DROSERA SPATHULATA* FROM *IN VITRO* CULTURES

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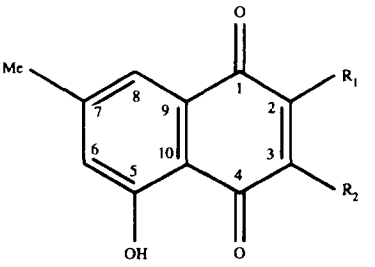
Key Word Index—*Drosera spathulata*; Droseraceae; 1,4-naphthoquinones; 2- and 3-methoxy-7-methyljuglones; 7-methyl-1, 4, 5-trihydroxynaphthalene 4-*O*-glucoside; rossoliside.

Abstract—From *Drosera spathulata* obtained by *in vitro* culture the known 1,4-naphthoquinones—7-methyljuglone, artefactual 2- and 3-methoxy-7-methyljuglone, together with 7-methyl-1, 4, 5-trihydroxynaphthalene 4-*O*-glucoside (rossoliside) were isolated. The structure of the last-named compound, was substantiated by NMR correlation techniques. It was also shown that 7-methyljuglone can be very easily released from rossoliside.

INTRODUCTION

Drosera spathulata Labill. (Droseraceae) is a carnivorous species native to south-east Asia and Australia [1], and does not occur in Europe. However, it can be easily developed by *in vitro* culture on Reinert–Mohr [2, 3] or diluted MS medium [4]. Our previous investigation of this species from *in vitro* culture showed the presence of several flavonoids and 7-methyljuglone [5], the latter earlier found in the naturally grown plants [6]. In the present study, a search for further naphthoquinones in this species led to the isolation of 2- and 3-methoxy-7-methyljuglones (**3**, **2**) formed from 7-methyljuglone (**1**) by the known reaction [7] during extraction with methanol. The spectral features allowing discrimination of these isomeric compounds are described. Moreover, rossoliside (**4**), so far known only from *D. rotundifolia* [8], was also isolated, characterized by NMR correlation techniques and found to be a precursor of **1** formation in water-containing plant extracts.



	R_1	R_2
1	H	H
2	H	OMe
3	OMe	H

RESULTS AND DISCUSSION

The methanolic extract of the fresh plants was fractionated into water distillate and chloroform and water-soluble fractions. The chloroform fractions yielded **1**–**3**. Compound **1** was 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthoquinone) from EIMS, UV and ^{1}H NMR data [7]. Compounds **2** and **3** had the same formula $C_{12}H_{10}O_4$ (HREIMS) and very similar EIMS and ^{1}H NMR spectra, indicating that they were 2- and/or 3-methoxy derivatives of **1**, previously described [7]. However, the authors of ref. [7] could differentiate those compounds by comparison with synthetic samples, not by spectral methods like UV, EIMS or ^{1}H NMR. In the present work, the position of methoxyl groups in each **2** and **3** was determined by so far unpublished ^{13}C NMR spectra, from differences in shifts of carbonyl groups observed in comparison with those of **1** (Table 1). In the case of **2**, both carbonyls appeared at δ_C 184.31 and 184.27 — one can be ascribed to the C-1 position as in **1** (δ_C 184.5), while the other, ascribable to C-4, was moved upfield (from δ_C 189.7 in **1**), as a result of the electron-withdrawing effect of the methoxyl group present on the adjacent carbon atom (C-3). A similar effect occurs in the case of 3-chloroplumbagin or droserone (=3-hydroxy-plumbagin) (C-4 carbonyl at δ_C 181.6 [9] and 183.9 [10], respectively) in comparison with plumbagin (C-4 at δ_C 189.6 [11]). In turn, **3** showed unchanged shift for C-4 (δ_C 190.3), but the signal ascribable to C-1 carbonyl was shifted to δ_C 179.7 due to OMe present at C-2. The less reliable distinction may be made by ^{1}H NMR shifts of chelated hydroxyls [12], which are mostly concentration independent [13]. The smaller value δ_H 11.72 for **2** compared to δ_H 12.15 for **3** reflects the weaker hydrogen-bonding caused by the inductive effect of the oxygen

Table 1. ^1H and ^{13}C NMR spectral data for compounds 1–3 (in $CDCl_3$)

	1		2		3	
	C*	H	C	H	C	H
1	184.5		184.31†		179.7	
2	139.3	6.92 *s*	110.2	6.12 *q* (0.3)	160.9†	
3	138.8	6.92 *s*	160.3‡		109.5	6.07 *q* (0.3)
4	189.7		184.27†		190.3	
5	161.8		162.3‡		161.3†	
6	124.1	7.09 *m*	123.5	7.04 *dq* (1.5/0.6)	124.9	7.07 *dq* (1.5/0.6)
7	148.5		149.3		147.2	
8	120.5	7.44 *m*	120.4	7.45 *dq* (1.5/0.6)	120.9	7.50 *dq* (1.5/0.6)
9	131.7		131.8		130.8	
10	113.1		112.3		112.1	
7-Me	22.2	2.44 *t* (0.4)	22.3	2.43 *t* (0.6)	22.0	2.42 *t* (0.6)
2-OMe					56.6	3.91 *d* (0.3)
3-OMe			56.5	3.91 *d* (0.3)		
5-OH		11.87 *s*§		11.72 *s*§		12.15 *s*§

* Data taken from ref. [11].

†‡ Interchangable assignments within the spectrum.

§ Signal disappeared with D_2O.

Decoupling experiments: **2**: irradiation at δ_H 2.43 changed signals δ_H 7.45 and 7.04 to doublets, J = 1.5 Hz each; irrad. at δ_H 3.91 changed δ_H 6.12 to a singlet and vice versa; **3**: irradiation at δ_H 2.42 changed singals δ_H 7.50 and 7.07 to doublets, J = 1.5 Hz each; irrad. at δ_H 3.91 changed δ_H 6.07 to a singlet and vice versa.

substituent at C-3 in **2**. Moreover, compounds can be discriminated by EIMS, whereas ion *m/z* 175 in **2** is replaced by that of *m/z* 176 in **3**. Therefore, **2** is 3-methoxy-7-methyljuglone, and **3** its 2-methoxy isomer, both previously reported as artefacts produced from **1** in the course of extensive extraction of plant material with methanol [7]. The same is true of *D. spathulata*, as **2** and **3** could not be detected after brief extraction with either methanol or acetone.

In the present study, the aqueous fraction freed from **1** by thorough chloroform extraction, when kept for several days at room temperature, showed again the presence of **1** (co-TLC). The precursor of **1** was a compound appearing on cellulose 2D-TLC (BAW, 15% HOAc) as a blue spot under UV_{365}, which, after detection with $AlCl_3$, gave a similar colour to that of 7-methyljuglone, i.e. pink–red in both UV and visible light. The same compound was detected by TLC in our previous investigations [5]. To avoid risk of decomposition, this compound (**4**) was isolated by chromatography on alumina. Compound **4** was hydrolysed rapidly by β-glucosidase to give **1** (co-TLC). This suggested that **4** is the glucoside of an unstable 7-methylhydrojuglone (7-methyl-1,4,5-trihydroxynaphthalene), which can be spontaneously oxidized by oxygen in air to the corresponding quinone (**1**). Such an assumption was confirmed by the ^1H NMR spectrum of **4** (Table 2), which revealed a set of signals with coupling patterns as in **1**, except that signals assignable to H-2 and H-3 were non-equivalent (δ_H 6.71 and 7.12), showing *ortho*-coupling (J = 8.3 Hz). There were two phenolic hydroxyls (δ_H 9.81 and 9.31) and one β-linked sugar residue, identified as glucopyranose from the ^{13}C NMR spectrum (Table 2). The resonances of directly hydrogen-bonded carbon atoms were determined from the HMQC spectrum. The position of the glucosyl at C-4 followed from the HMBC spectrum on the basis that the carbon δ_C 146.6 involved in glucosidic linkage due to correlation with anomeric proton of glucosyl (δ_H 4.84) must correspond to this position, as it also correlated with *ortho*-related protons, i.e. H-2 and H-3 only. If the glucosyl was at the alternative C-1 or C-5 position, the HMBC spectral correlations of those carbons signals with either of the *meta*-related protons H-8 or H-6, respectively (δ_H 7.37, 6.64) could be expected. The further HMBC correlations observed allowed assignments of the remaining NMR resonances.

Hence, **4** is 7-methyl-1,4,5-trihydroxynaphthalene 4-*O*-β-glucoside (=rossoliside). Rossoliside was described so far only from *D. rotundifolia* (Droseraceae) as early as in 1971 [8], but it could not be found in this species in more recent investigations [14]. From the isolation procedure described in ref. [14], one suspects that this compound even if present could be lost due to its lability in aqueous solutions. Compound **4** can be considered as a combined form of 7-methyljuglone, in a similar way as 1,4,5-trihydroxynaphthalene glucoside is for juglone in *Juglans regia* (Juglandaceae) [15]. The same is true of hydroplumbagin 4-*O*-glucoside from *Dionaea muscipula* (Droseraceae) [9].

EXPERIMENTAL

Plant material. Fully developed plants obtained by *in vitro* culture on Reinert–Mohr medium in a similar way

Table 2. ^1H and ^{13}C NMR data for **4*** (in DMSO d_6)

	C	H
1	148.3	
2	107.3	6.71 *d* (8.3)
3	110.7	7.12 *d* (8.3)
4	146.6	
5	153.0	
6	112.6	6.64 *br d* (1.7)
7	135.3	
8	112.4	7.37 *dq* (1.7 ~0.5)
9	126.9	
10	114.3	
7-Me	21.3	2.37 *br s*
1-OH		9.81 *br s*
5-OH		9.31 *s*
1′	103.6	4.84 *d* (7.8)
2′	73.5	3.35 *m*
3′	76.3	3.35 *m*
4′	69.8	3.21 *t* (9)
5′	77.5	3.35 *m*
6′	60.7	3.76 *dd* (1.5 12)
		3.52 *dd* (6 12)

Sugar OHs: 5.77 *d* (5.1), 5.28 *d* (4.6), 5.18 *d* (5.4), 4.75 *t* (6.0) (signals exchangeable with D_2O).

*Assignments aided with HMQC and HMBC.

as described in ref. [5] at the Botanical Garden, University of Wrocław, Poland, were collected in May (283 g) and October (450 g) 1992 and July 1994 (150 g) (collections A, B and C, resp.).

Spectroscopy. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra recorded with TMS as int. standard. HMBC spectra were measured for $J_{HC} = 7$ or 4 Hz. UV spectra made according to ref. [16].

Extraction and isolation. The whole fresh plants (separately from A and B collections) were plunged into hot MeOH followed by maceration at room temp., further repeated ×2 (2 months). The combined extracts were concd at 40°, collecting the residual water separately, as a yellow distillate containing **1** [5]. The dry extracts were each partitioned between H_2O and $CHCl_3$. The $CHCl_3$ frs corresponding to both materials were identical by TLC and thus combined and chromatographed by silica gel CC with toluene to give frs containing **1** and **2** + **3**. Subsequent prep. TLC (toluene–HCOOH, 99:1) gave **1** (R_f 0.37) (after 1 development) and **2** and **3** (3 developments) (R_f 0.28, 0.36). Compound **2** was purified from the unidentified non-phenolic compound (R_f 0.38) by TLC in toluene–EtOAc (4:1) (R_f 0.50). The material of collection C was divided into three equal portions, two being immersed in MeOH (C1, C2) and the third in Me_2CO (C3). After 2 days, extracts of C1 and C3 were concd and partitioned as above. The $CHCl_3$ frs showed no **2** or **3** (co-TLC). The H_2O frs, after 5 days at room temp., showed the presence of **1** (co-TLC). They were extd with *n*-BuOH (×3), concd and kept dry. The extraction of C1–C3 was continued (×2) (3 months) and extracts processed separately to obtain $CHCl_3$ and *n*-BuOH frs. The $CHCl_3$ fr. of C2 contained **2** and **3** (co-TLC). Combined *n*-BuOH frs were subjected to CC on neutral alumina (POCh) in 50% EtOH to afford crude **4** (60 mg). The final clean-up of **1**–**4** was by CC on Sephadex LH-20 (Pharmacia) in MeOH. Yields: **1** (4 mg), **2** (4 mg), **3** (6 mg), **4** (50 mg).

7-Methyljuglone (**1**). EIMS *m/z* (rel. int. %): 188 (100) $[M]^+$, 173 (5), 160 (11), 134 (15), 132 (19), 131 (15). ^1H and ^{13}C NMR: Table 1.

5-Hydroxy-3-methoxy-7-methyl-1,4-naphthoquinone (**2**). Orange red crystals. TLC: dark-yellow spot under UV_{365}. EIMS *m/z* (rel. int. %): 218 $[M]^+$ (100), 203 $[M-Me]^+$ (26), 190 $[M-CO]^+$ (17), 189 $[M-HCO]^+$ (45), 188 $[M-2Me]^+$ (19), 187 $[M-OMe]^+$ (19), 175 $[M-Ac]^+$ (6), 161 $[M-2HCO]^+$ (4), 160 (8), 147 (14), 132 (9), 119 (25), 103 (4), 91 (5), 77 (8), 65 (5). HREIMS *m/z* found: 218.05767 $[M]^+$ ($C_{12}H_{10}O_4$ req. 218.05784), 203.03389 $[M-Me]$ ($C_{11}H_7O_4$ req. 203.03438), 189.05349 $[M-HCO]$ ($C_{11}H_9O_3$ req. 189.05511), 175.03845 $[M-MeCO]$ ($C_{10}H_7O_3$ req. 189.05511). UV λ_{max}^{MeOH} nm: 214, 246, 288, 413; +NaOMe 288, 512; +NaOAc 288, 456*sh*, 519; $+H_3BO_3$/NaOAc 246*sh*, 289, 415; $+AlCl_3$ 244*sh*, 298, 492; $+AlCl_3$ +HCl as with $AlCl_3$.

5-Hydroxy-2-methoxy-7-methyl-1,4-naphthoquinone (**3**). Orange crystals. TLC: brown spot under UV_{365}. EIMS (*m/z*) (rel. int. %): 218 $[M]^+$ (100), 203 $[M-Me]^+$ (18), 190 $[M-CO]^+$ (17), 189 $[M-HCO]^+$ (45), 188 $[M-2Me]^+$ (31), 187 $[M-OMe]^+$ (19), 176 $[M-CH_2CO]^+$ (9), 161 $[M-2HCO]^+$ (14), 147 (20), 132 (16), 119 (30), 103 (8), 91 (8), 77 (15), 65 (5). HREIMS *m/z* found: 218.05787 $[M]^+$ ($C_{12}H_{10}O_4$ req. 218.05784), 203.03408 $[M-Me]^+$ ($C_{11}H_7O_4$ req. 203.03438), 189.05151 $[M-HCO]^+$ ($C_{11}H_9O_3$ req. 189.05511), 176.04342 $[M-CH_2CO]^+$ ($C_{10}H_8O_3$ req. 176.04729). UV λ_{max}^{MeOH} nm: 214, 246, 288, 422; +NaOMe 288, 519; +NaOAc 246*sh*, 288, 427, 520; $+H_3BO_3$/NaOAc 246*sh*, 289, 424; $+AlCl_3$ 248, 297, 494; $+AlCl_3$ + HCl as with $AlCl_3$.

7-Methyl-1,4,5-trihydroxy-naphthalene 4-O-β-glucopyranoside (rossoliside) (**4**). Beige amorphous powder. 2D-TLC on cellulose, pre-coated (Merck) in BAW and 15% HOAc, R_f 0.69, 0.52, resp., LSI-MS (+ive) *m/z* (%): 357 $[M+Na]^+$ (51), 353 $[M+H]^+$ (16), 352 $[M]^+$ (11), 190 $[M-glc]^+$ (100). UV λ_{max}^{MeOH} nm: 226, 294*sh*, 307, 326, 345; +NaOMe 327*sh*, 343, 354; no shifts with NaOAc, H_3BO_3 + NaOAc, $AlCl_3$ and $AlCl_3$ + HCl.

Enzymic hydrolysis. Soln of **4** (1 mg) and β-glucosidase (Sigma) (0.5 mg) in 1 ml H_2O covered with 0.5 ml toluene was kept at room temp. It became yellow in 3 min and the pigment (**1**, co-TLC) migrated into the toluene layer. Hydrolysis in pure H_2O: as above, but without enzyme. H_2O layer became increasingly brownish after 1 week [several products besides **4** by TLC on polyamide 6D (Riedel de Haehn) in H_2O–*n*-BuOH–Me_2CO (16:3:3)], while toluene layer was clear, but contained **1** (co-TLC) after further 3 weeks.

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REFERENCES

1. Juniper, D. E., Robins, R. J. and Joel, D. M. (1989) *The Carnivorous Plants*, Academic Press, London.
2. Kukułczanka, K. (1991) *Botan. Gardens, Microprop. News (Kew)* **1**, 37.
3. Kukułczanka, K. and Czastka, B. (1991) *Prace Ogr. Botan. PAN* **1**, 55.
4. Bobak, M., Blehova, A., Samaj, J., Ovecka, M. and Kristin, J. (1993) *J. Plant Physiol.* **142**, 215.
5. Budzianowski, J., Skrzypczak, L. and Kukułczanka, K. (1993) *Acta Hortic.* **330**, 277.
6. Zenk, M. H., Fürhringer, M. and Steglich, W. (1969) *Phytochemistry* **8**, 2199.
7. Tezuka, M., Kuoryanagi, M., Yoshihira, K. and Natori, S. (1972) *Chem. Pharm. Bull.* **20**, 202.
8. Sampara-Rumantir, N. (1971) *Pharm. Weekbl.* **106**, 653.
9. Kreher, B., Neshmelyi, A. and Wagner, H. (1990) *Phytochemistry* **29**, 605.
10. Schölly, T. and Kapetanidis, I. (1989) *Pharm. Acta Helv.* **64**, 66.
11. Sankaram, A. V. B., Reddy, V. V. N. and Marthandamurthi, M. (1986) *Phytochemistry* **25**, 2867.
12. de Oliveira, A. B., Ralsan, D. S., de Oliveira, G. G. and Maia, J. G. S. (1993) *Phytochemistry* **34**, 1409.
13. Lillie, T. J. and Musgrave, O. C. (1977) *J. Chem. Soc., Perkin I*, 355.
14. Schölly, T. and Kapetanidis, I. (1989) *Planta Med.* **55**, 611.
15. Binder, R. G., Benson, M. E. and Flath, R. A. (1989) *Phytochemistry* **28**, 2799.
16. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.