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NAPHTHOHYDROQUINONE GLUCOSIDES OF *DROSERA ROTUNDIFOLIA* AND *D. INTERMEDIA* FROM *IN VITRO* CULTURES

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Key Word Index—*Drosera rotundifolia*, *D. intermedia*; Droseraceae; naphthoquinones; 1,4-naphthohydroquinone glucosides; rossoliside; hydroplumbagin glucoside; plumbagin; 7-methyljuglone.

Abstract—Rossoliside (7-methylhydrojuglone 4-*O*-glucoside) was isolated from *Drosera rotundifolia*, together with hydroplumbagin 4-*O*-glucoside, from *D. intermedia*, both of which were produced by *in vitro* micropropagation. Hydroplumbagin glucoside released the corresponding 1,4-naphthoquinone (plumbagin) more rapidly than rossoliside (7-methyljuglone). These glucosides can be detected in plant extracts by reversed-phase TLC and appearance of the corresponding free quinones after treatment with β-glucosidase.

INTRODUCTION

Recently, we found that rossoliside (7-methylhydrojuglone 4-*O*-glucoside) (**1**) isolated from *Drosera spathulata* obtained by *in vitro* culture can release 7-methyljuglone (**3**) by cleavage of the glucosidic bond followed by air oxidation of the aglycone not only on β-glucosidase hydrolysis, but also in pure water or in water containing plant extract [1]. The bound form of plumbagin (**4**), i.e. hydroplumbagin 4-*O*-glucoside (**2**) has been obtained from *Dionaea muscipula* (Droseraceae) [2]. It was of interest, therefore, to determine whether the other Droseraceae species known to contain free plumbagin and/or 7-methyljuglone [3, 4] contain significant amounts of the aforemention glucosides. The present investigation was carried out with *D. rotundifolia*, known as a producer of mainly 7-methyljuglone [5], and with *D. intermedia*, a producer of mainly plumbagin, grown naturally [5] or produced from *in vitro* cultures [5, 6]. *D. rotundifolia* is probably the most important medicinally *Drosera* species and is used for the treatment of respiratory diseases (e.g. whooping cough) [7]; the activity is attributed to plumbagin and 7-methyljuglone [8]. The plant material for the present investigations was obtained by *in vitro* micropropagation. The isolation procedure used for the search for those unstable naphthohydroquinone glucosides (**1**, **2**) was similar to that already applied in the case of *D. spathulata* [1].

RESULTS AND DISCUSSION

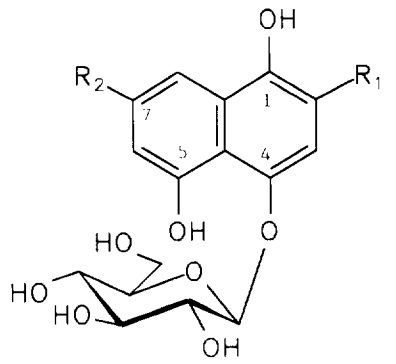
The methanolic extracts of the fresh plants were each processed to obtain a distillate of residual water and chloroform, butanol and water fractions. The butanolic solubles yielded pure naphthohydroquinone glucoside samples by neutral alumina and Sephadex LH-20 column chromatography.

The glucoside isolated from *D. rotundifolia* was pure rossoliside (**1**) as shown by direct comparisons with an authentic sample and β-glucosidase hydrolysis to 7-methyljuglone (**3**) [1]. The presence of **1** correlated well with the co-occurrence of only free compound **3** (distillate, chloroform fraction, co-TLC).

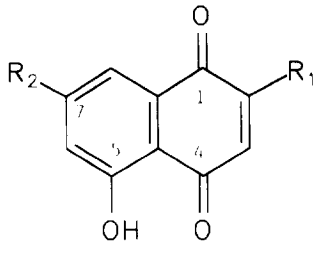
The glucoside sample from *D. intermedia*, in contrast to the expectation from the co-occurrence in this plant of mainly free plumbagin (**4**) and only traces of 7-methyljuglone (**3**) (distillate, chloroform fraction, co-TLC), surprisingly appeared to be mainly rossoliside (**1**) with only a small admixture of hydroplumbagin 4-*O*-glucoside (**2**), as shown by enzymic hydrolysis to compounds **3** and **4** and the ^{1}H and ^{13}C NMR spectra [1, 2] (the ratio **1**:**2** was 7:1 from ^{1}H NMR integrals). However, the aqueous solution of this mixture (**1** + **2**) yielded plumbagin (**4**) first (after 6 days, co-TLC), which indicated hydroplumbagin glucoside (**2**) to be more susceptible to hydrolysis and explained its lower contribution in the sample and higher proportion of the free plumbagin over 7-methyljuglone in the extract.

The glucosides **1** and **2** can be detected in butanolic fractions or crude extracts by reversed-phase TLC and the appearance of the corresponding free quinones (**3**, **4**) after treatment with β-glucosidase (see Experimental).

Rossoliside (**1**) was isolated many years ago from naturally grown *D. rotundifolia* [9] but could not be



	R_1	R_2
1	H	CH_3
2	CH_3	H



	R_1	R_2
3	H	CH_3
4	CH_3	H

found in this plant in a more recent study [10], probably for the reasons raised in ref. [1]. Hydroplumbagin glucoside (**2**) was also reported from this species [11], although the position of glucosidic linkage was subsequently revised from C-5 to C-4 [2]. The present study showed the absence of this compound (**2**) in *D. rotundifolia* which correlates with the absence of plumbagin (**4**). It has been reported, that wild-growing *D. rotundifolia* plants contain both free plumbagin and 7-methyljuglone (**3**), while those obtained by *in vitro* culture on Murashige–Skoog (MS) medium contain only 7-methyljuglone [5]. Similar observations [5] made with *D. intermedia* showed only quantitative differences in the content of both quinones.

In the case of *D. intermedia*, the presence of hydroplumbagin glucoside has been suggested [11], but rossoliside was isolated for the first time in this study.

EXPERIMENTAL

Plant material. Fully developed *D. rotundifolia* was obtained by *in vitro* culture: (1) by multiplication from leaf rosettes on half-strength MS medium without hormones [12, 13], (2) by culture on a Reinert–Mohr medium in a similar way to that described in refs [12, 13] Botanical Garden, University of Wrocław, Poland). The plant material was harvested in October 1994 (200 g) and August 1995 (46 g) (batches A and B, respectively). *D. intermedia* was obtained on a Reinert-Mohr medium as above [6, 12, 13] and collected in June 1992 (18 g) and February 1995 (380 g) (batches A and B, resp.).

Extraction and isolation. Whole fresh plants were extracted with MeOH (×2) (*D. rotundifolia*, batch A, 1 week; *D. intermedia*, batch B, 1 month) (or ×1) (*D. rotundifolia*, batch B, 3 days) and the extracts processed to obtain a distillate of residual water, and $CHCl_3$, *n*-BuOH and H_2O frs as described for *D. spathulata* [1], except that fractionation was performed immediately after concn of the MeOH extracts. *D. intermedia*, batch A, was extracted with MeOH (×3) within 10 days and the extract sepd into distillate, $CHCl_3$ and H_2O frs. The toluene extracts of distillates and $CHCl_3$ frs showed the presence of plumbagin (**4**) and traces of 7-methyljuglone (**3**) in *D. intermedia* (batches A and B) or only **3** in *D. rotundifolia* (batches A and B) by co-TLC on silica gel (pre-coated, Merck) in toluene–HCOOH (99:1) [2]. Both distillate and $CHCl_3$ fr. of *D. intermedia*, batch A, on prep. TLC in toluene, yielded **3** and **4** (co-TLC, UV [1]), which were detected previously in this species [6]. Plumbagin (**4**) was identified by UV, EI-MS [5], ^1H and ^{13}C NMR [14, 15] and co-TLC. We report its ^1H NMR data because of an interesting long-range coupling between 5-OH and H-7 observed in the spectrum recorded at 300 MHz (in $CDCl_3$). This coupling disappeared after addition of D_2O. ^1H NMR: δ 11.97 (1H, *d*, $J = 0.4$ Hz, HO-5), 7.64 (1H, *dd*, $J = 7.5/2.2$ Hz, H-8), 7.60 (1H, *td*, $J = 7.5/0.4$ Hz, H-7), 7.25 (1H, *dd*, $J = 7.5/2.2$ Hz, H-6), 6.81 (1H, *q*, $J = 1.6$ Hz, H-3), 2.19 (1H, *d*, $J = 1.6$ Hz, Me-2).

n-BuOH frs were chromatographed as that of *D. spathulata* [1] to yield rossoliside (**1**) (79 mg) from *D. rotundifolia* (batch A) and a mixt. of **1** and hydroplumbagin 4-*O*-glucoside (**2**) (213 mg) from *D. intermedia* (batch B). **1** + **2** showed in its NMR spectra (^1H: 300 MHz; ^{13}C: 75 MHz, in DMSO-d_6) two sets of signals for each component (well-resolved except for H-6 of an aglycone and C-2 to C-6 of glucosyls) in strict accord with previously reported data [1, 2].

Hydrolysis procedures. These were performed in a biphasic toluene–H_2O system with and without β-glucosidase as described in ref. [1].

TLC. On C-18 reversed-phase plates (RP-18, precoated, Merck) in MeOH–H_2O (1:1) **1** (blue under UV 365) and **2** (blue-violet under UV 365 nm) had R_f 0.38 and 0.48, respectively. Both became visible in daylight after several hours owing to browning as a result of decomposition on exposure to air and gave a red colour under UV 365 nm and in daylight after spraying with 1% $AlCl_3$ in EtOH followed by heating. The position of each compound on the chromatogram was ascertained by sepn of a 3 mg sample of **1** + **2**, elution of the bands from the still wet plate (MeOH) and subsequent β-glucosidase hydrolysis to give either **3** or **4**. Analysis was applicable to crude extracts. Normal-phase sys-

tems, including 2D-TLC in 1-BuOH–HOAc–H_2O (4:1:5) (BAW) and HOAc–H_2O (3:17) (15% HOAC) [1] were unable to separate both glucosides.

Detection of naphthohydroquinone glucosides in a crude plant extract. A 5 ml sample of the H_2O phase of the extract was washed with $CHCl_3$ and toluene, treated with 2 mg β-glucosidase (Sigma), covered with 0.5 ml toluene and after standing at room temp. (4 hr), shaken and the toluene layer (yellow) analysed for the presence of quinones (**3**, **4**) (co-TLC as above).

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