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2-METHYLNAPHTHAZARIN 5-*O*-GLUCOSIDE FROM THE METHANOL EXTRACTS OF *IN VITRO* CULTURES OF *DROSERA* SPECIES

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Key Word Index—*Drosera rotundifolia*; *D. spathulata*; Droseraceae; naphthoquinones; 5,8-dihydroxy-2-methyl-1,4-naphthoquinone 5-*O*-glucoside; 2-methylnaphthazarin 5-*O*-glucoside; rossoliside.

Abstract—The methanol extracts of *Drosera rotundifolia* and *D. spathulata* obtained by *in vitro* micropropagation yielded the new pigment 2-methylnaphthazarin (5,8-dihydroxy-2-methyl-1,4-naphthoquinone) 5-*O*-glucoside, which is an artefact formed from rossoliside (7-methylhydrojuglone 4-*O*-glucoside) during extraction with methanol. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

In our previous communication on phenolics in some species of the genus *Drosera* obtained by *in vitro* micropropagation on Reinert–Mohr medium, we have mentioned the isolation of the 5-(or 8)-*O*-glucoside of 8-hydroxyplumbagin (**1**), i.e. of 5,8-dihydroxy-2-methyl-1,4-naphthoquinone (= 2-methylnaphthazarin, = ramentone) from *D. rotundifolia* L. but analytical details were not presented [1].

More recently, the naphthohydroquinone glucoside - rossoliside (i.e. 7-methylhydrojuglone 4-*O*-glucoside, = 7-methyl-1,4,5-trihydroxy-naphthalene 4-*O*-glucoside) was obtained from this plant [2].

In the present paper, compound **1** was finally determined as 2-methylnaphthazarin 5-*O*-glucoside and shown to be an artefact formed from rossoliside (**3**) during extraction with methanol (but not acetone).

RESULTS AND DISCUSSION

The methanol extract of the fresh plants of *D. rotundifolia* was fractionated into tissue water and chloroform and water fractions. The latter, upon polyamide column chromatography and preparative TLC, gave compound **1**.

Compound **1**, in its ^{1}H NMR spectrum showed a C-methyl group (δ_H 2.08) coupled to a vinylic proton (δ_H 6.86) both assigned to the 1,4-quinonoid ring, the carbonyls of which were evident from ^{13}C NMR spectrum (δ_C 190.4 and 183.4). There were also a chelated hydroxyl group (δ_H 12.45), two *ortho*-related protons (δ_H 7.69, 7.35) and an anomeric proton (δ_H 4.94, *d*) of a β-*O*-linked (J = 7 Hz) glucopyranosyl (^{13}C NMR); all were being placed on the benzenoid ring of the 1,4-naphthoquinone. Unfortunately, decomposition of compound **1** after NMR measurements in DMSO-d_6 solution prevented any further structural elucidation. The glucosyloxy group was placed at the second peri position to the carbonyl group (C-1 or C-4), as the aglycone of compound **1** obtained on the trial isolation from *D. spathulata* Labill., which was available to us in larger amounts, appeared to be 5,8-dihydroxy - 2 - methyl - 1,4 - naphthoquinone (= 2 - methyl-naphthazarin, ramentone) (**2**). The final placement of the glucosidic linkage at C-5, not the alternative C-8 position, followed from the observation that compound **1** appeared in the samples of rossoliside (**3**) kept in open vessels and redissolved several times in methanol, e.g. for TLC. Hence, in the ^{13}C NMR spectrum of compound **1** the carbonyl resonating at δ_C 190.4 is assigned to the C-1 position and its more upfield shift compared with that of C-4 (δ_C 183.4) is explained by chelation of the OH at C-8 (peri-OH) analogous to that of juglone (5-hydroxy-1,4-naphthoquinone) [4]. It is unlikely that compound **1** could pass through the alumina columns employed for the isolation of compound **3** [2, 3], because it irreversibly adsorbs on this material. Thus, there must be oxidation at C-8 (and C-5) of rossoliside (**3**) to the corresponding 1,4-quinone, i.e. 5,8-dihydroxy-2-methyl-1,4-naphthoquinone 5-*O*-glucoside (**1**), which to the best of my knowledge is a new product. The scheme of the general turnover of rossoliside (**3**) in water–methanol extracts, based on the present and earlier studies [2, 3], is shown in Fig. 1.

The contact of rossoliside (**3**) with methanol must be important for the formation of compound **1** as it was present (TLC, see Experimental) in methanol extracts, but barely detectable in acetone extracts of *D. spathulata* (contains rossoliside) obtained in a previous

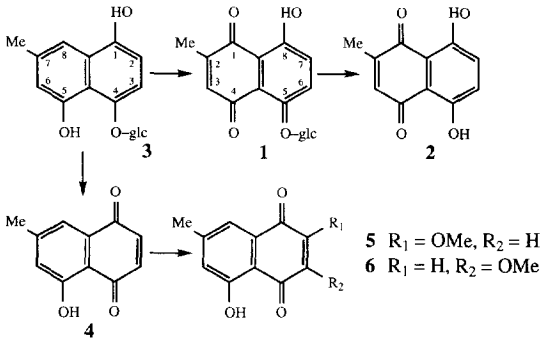

Fig. 1. Turnover of rossoliside (**3**) in water–methanol extracts.

study [3]. Moreover, compound **1** was absent from the methanol extract of another Droseraceae plant, *Dionaea muscipula*, which completely lacks 7-methyljuglone [1, 5] (i.e. rossoliside occurence was unlikely, as it releases 7-methyljuglone in extracts [2, 3]).

EXPERIMENTAL

Plant material. Fully developed *D. rotundifolia* was obtained by *in vitro* micropropagation on a Reinert–Mohr medium similar to refs [6, 7] at the Botanical Garden, University of Wrocław, Poland. It was collected in January 1990 (190 g). *Dionaea spathulata* obtained in the same manner was collected in May (283 g) and October (475 g) 1992 and in September 1993 (756 g).

TLC. Silica gel (Merck) in toluene–EtOH (4:1) (sys. 1), or toluene–HCO_2H (99:1) (sys. 2); on polyamide 6MN (Macherey–Nagel) in H_2O–*n*-BuOH–Me_2CO 16:3:3 (sys. 3) or H_2O (sys. 4). 2D TLC on cellulose (pre-coated, Merck) in BAW (*n*-BuOH–HOAc–H_2O, 4:1:5) and 15% HOAc (HOAc–H_2O, 3:17) (sys. 5).

Extraction and isolation. The whole fresh plants of *D. rotundifolia* were plunged into hot MeOH and macerated (×3) at room temp. for 10 months. The extract was concd collecting the residual water separately as a yellow soln containing 7-methyljuglone (**4**) [3]. The dry extract was partitioned between H_2O and $CHCl_3$. The H_2O fr. was sepd by polyamide CC (SC-6, Macherey–Nagel) with H_2O, H_2O–*n*-BuOH (100:9), H_2O–*n*-BuOH-Me_2CO (10:1:1, 10:2:2), MeOH and 0.01% NH_4OH–MeOH, respectively. The visible burgundy band eluted with H_2O after prep. TLC (pre-coated, silica gel, Merck) in toluene–EtOH, (4:1 (sys. 1) and elution of the band *Rf* 0.24 and crystallization from the same solvent gave compound **1** (8 mg). To obtain more compound **1** each collection of *D. spathulata* was processed in the same way and the H_2O frs were evapd and kept dry in a refrigerator before they were combined and sepd by polyamide CC with H_2O to give burgundy band A containing compound **1** (coTLC). The main fr. of band A upon concn changed colour to purple–red and the pigment formed was recovered by extraction with toluene and prep. TLC (toluene) and identified as 2-methylnaphthazarin (**2**) (^{1}H NMR [8], UV [9]), while compound **1** dissappeared. The fr. corresponding to the trailing edge of the band A still contained compound **1**, which was first extracted with *n*-BuOH before concn to avoid risk of hydrolysis, and then purified by prep. TLC (sys. 1). It was identical to the sample from *D. rotundifolia* (UV, coTLC in sys. 1-5).

5,8-Dihydroxy-2-methyl-1,4-naphthoquinone 5-O-β-glucoside (**1**). Dark-purple crystals (toluene–EtOH, 4:1). TLC appearance: UV_{365}: brown, after spraying with $AlCl_3$ [2] fluorescent pink; day-light: orange, with $AlCl_3$ pink. R_f (×100): 17, 0, 73, 33, 70/83 in sys. 1–5, resp. UV λ_{max}^{MeOH} nm: 265, 449; +NaOMe 271, 351, 544; +NaOAc 267, 460, 510; +NaOAc–H_3BO_3 as in MeOH; +$AlCl_3$ 272, 505sh, 534, 574sh; + $AlCl_3$–HCl 278, 505sh, 534, 575. ^{1}H NMR (300 MHz, DMSO-d_6, TMS): δ 12.45 (1H, *s*, 5-OH), 7.69 (1H, *d*, J = 9.6 Hz, H-6), 7.35, (1H, *d*, J = 9.6 Hz, H-7), 6.86 (1H, *q*, J = 1.5 Hz, H-3), 4.94 (1H, *d*, J = 7.2 Hz, H-1′ of glucosyl), 2.08 (3H, *d*, J = 1.5 Hz, 2-CH_3). ^{13}C NMR (75 MHz, DMSO-d_6, TMS): δ [aglycone] 190.4 (C-1), 183.1 (C-4), 156.5 (C-8), 150.6 (C-5), 145.7 (C-2), 137.9 (C-3), 128.2 (C-7), 125.8 (C-6), 118.2 (C-9), 114.1 (C-10), 14.8 (2-CH_3); [glucosyl] 101.6 (C-1), 73.3 (C-2), 76.1 (C-3), 69.5 (C-4), 77.1 (C-5), 60.6 (C-6).

Compound **2**. UV λ_{max}^{MeOH} nm: 273, 480, 510, 543sh; +NaOMe 273, 300sh, 530sh, 576, 613; NaOAc 273, 510sh, 572, 613; + $AlCl_3$ 309, 322sh, 340sh, 495, 532, 574 ; +$AlCl_3$–HCl as in $AlCl_3$. ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 12.57 (1H, *s*, 5-OH), 12.46 (1H, *s*, 8-OH), 7.21 (2H, *s*, H-6, H-7), 6.91 (1H, *q*, J = 1.2 Hz, H-3), 2.24 (1H, *d*, J = 1.2 Hz, 2-CH_3).

Formation of compound **1** *from rossoliside* (**3**). The impure leading edge of the Sephadex LH-20 column band containing compound **3** plus hydroplumbagin glucoside from *D. intermedia* [2] (23 mg) was kept in an unsealed round-bottom flask (100 ml) for 7 months. It was dissolved several times in MeOH for TLC examination and then left each time for the solvent to evaporate. The solid became brown and contained compound **3**, plumbagin, 7-methyljuglone (**4**) and compound **1** by coTLC in sys. 1 and 2. Compound **1** (0.5 mg) was isolated after successive CC on polyamide (H_2O), prep. TLC (sys. 1) and Sephadex LH-20 CC (MeOH). Samples of compound **3** from *D. rotundifolia* [2], *D. spathulata* [3] and *D. intermedia* (with admixture of hydroplumbagin glucoside) [2], each *ca* 2 mg, kept in open vials and used for TLC in MeOH solns showed the presence of compound **1** (coTLC, sys. 1, 3). They were each hydrolysed with β-glucosidase according to [3] to give compound **2** besides 7-methyljuglone (**4**) and plumbagin (*D. intermedia* only) (coTLC in sys. 2, R_f: 0.52, 0.42, 0.50, resp.).

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