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THE IDENTIFICATION OF ORCINOL IN HIGHER PLANTS IN THE FAMILY ERICACEAE

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Abstract—Orcinol (5-methylresorcinol) has been detected for the first time in higher plants, in ten species of the Ericaceae. It was found as the β-D-glucoside in leaves of *Erica arborea* var. *alpina* and identified by comparison with material synthesized by feeding broad beans with free orcinol. Besides occurring in *E. arborea*, orcinol was detected in all of three *Phyllodoce* species examined, in two *Pieris* species and in *Rhododendron* (in four of fifty-five species surveyed). It is thus of rare but scattered occurrence in the family.

INTRODUCTION

AS PART of a chemotaxonomic survey of phenolics and flavonoids[1,2] in the Ericaceae, the phenolic acids present in acid-hydrolysed leaf extracts were examined by two-dimensional TLC on silica gel and cellulose. During this work, a novel phenol was detected as a minor constituent in *Phyllodoce* and several other species. It had the mobility on chromatoplates of a dihydroxybenzene and its colour reactions (red colour with vanillin–HCl) indicated that it was *m*-disubstituted or based on resorcinol, rather than *o*- or *p*- disubstituted (i.e. catechol or hydroquinone derived). In view of the rarity of simple phenols in higher plants (according to Karrer,[3] resorcinol has never been found free in plants) and of the taxonomic interest of this substance, it was necessary to identify it more fully. This is reported in the present paper.

RESULTS

Although first detected in acid-hydrolysed leaf extracts of *Phyllodoce*, none of the three species of this genus containing the novel phenol was available in sufficient quantities for isolation. Further surveys revealed that *Erica arborea*, a large woody shrub, was also a good source and this plant was used for large-scale extraction. Because of the excessive amounts of other phenolics present in the leaves, there were considerable difficulties in isolation and purification and less than milligram quantities were available for identification. The material so obtained (see Experimental) was very similar in spectrum and colour reactions to resorcinol and the R_fs were very close but not identical (Table 1). Examination of the mass spectrum of a crude isolate showed a peak at 124 (corresponding to the molecular weight of methylresorcinol) so the unknown was directly compared with 2-, 4- and 5-methylresorcinols. These three isomers can be distinguished by colour reactions, by spectral and chromatographic means (Table 1) and the unknown was readily identified as the 5-methyl isomer (i.e. orcinol) by these procedures.

[1] J. B. HARBORNE, *Phytochem.* **8**, 177 (1969).
[2] J. B. HARBORNE, *Phytochem.* **8**, 419 (1969).
[3] W. KARRER, *Konstitution und Vorkommen der Organischer Pflanzenstoffe*, Birkhauser Verlag, Basel (1958).

Direct leaf extracts of *Erica* were then examined to see if orcinol was present in combined form as a glycoside. All attempts to separate an orcinol glycoside from these extracts by paper or thin-layer chromatography failed because of the large amounts of cinnamic acid esters and tannins present, but paper electrophoresis at pH 8·8 clearly separated a glycoside with the right colour and spectral properties. Purified material was rapidly hydrolysed by acid or β-glucosidase to orcinol and glucose, thus showing that orcinol β-D-glucoside (I) was present. This substance was synthesized by feeding orcinol to broad beans, using Pridham and Saltmarsh's procedure,[4] and it proved to be identical in every way with the natural material (Table 1).

TABLE 1. CHROMATOGRAPHIC, SPECTRAL AND COLOUR PROPERTIES OF ORCINOL, ITS β-GLUCOSIDE AND RELATED PHENOLS

Phenol	R_f (×100) in solvent* 1	2	3	4	Vanillin–HCl colour†	λ_{max}^{EtOH}	λ_{max}^{NaOEt}
Erica aglycone‡	19	62	46	67	Bluish pink	276, 282	294
Orcinol	19	62	46	67		276, 282	294
4-Methylresorcinol	25	63	59	65	Brick red	282	291
2-Methylresorcinol	40	64	58	73	Bluish pink	275, 280	288
Resorcinol	17	59	48	74	Red	276, 283	293
Catechol	35	66	58	72	None	279	decomp.
Hydroquinone	18	58	34	69	None	295	decomp.
	R_f (×100) in solvent 5	6	7	8			
Erica glucoside‡	60	77	73	49	Red (after heating)	273, 278	285
Orcinol glucoside	60	77	73	49		272, 278	288
Resorcinol glucoside	53	67	78	39		274, 279	288

* Solvent key: 1, HOAc–$CHCl_3$ (1:9); 2, EtOAc–benzene (9:11); 3, Benzene–MeOH–HOAc (45:8:4); 4, 6% HOAc; 5, butanol–HOAc–H_2O (4:1:5); 6, butanol–ethanol–water (4:1:2·2); 7, 15% HOAc; 8, EtOAc–HOAc–H_2O (4:1:5). Solvents 1 and 2 on silica gel, solvents 3–8 on MN 300 cellulose.

† All phenols gave a blue colour on spraying with Folin–Ciocalteau reagent and fuming with NH_3; both catechol and hydroquinone were distinguished by their giving an immediate blue colour before NH_3 treatment. The glucosides were detected by use of Gibbs reagent and NH_3; resorcinol glucoside gave a cobalt blue colour, orcinol glucoside a mauve colour.

‡ Natural and synthetic aglycone and glucoside were co-chromatographed and showed no separation. The glucosides were also co-electrophoresed without separation.

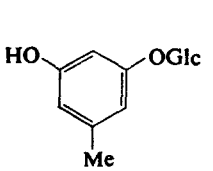
This appears to be the first report of a simple resorcinol derivative occurring in higher plants. Orcinol, however, is well known as a degradation product of lichen depsidones and has also been found itself in the lichen *Umbilicaria papulosa* by Miller *et al.*[5] It is also produced by several fungi[6–8] and is reported to be formed in orchid roots following microbial infection.[9] It is conceivable that its presence in *Erica* may be as a product of fungal symbiosis, since mycorrhiza are known to be associated with roots of the Ericaceae.[10] At present, this seems

[4] J. B. PRIDHAM and M. J. SALTMARSH, *Biochem. J.* **87**, 218 (1963).
[5] E. V. MILLER, E. G. CLAIBOURNE, T. SCHAEFERS and M. GORDON, *Botan. Gaz.* **126**, 100 (1965).
[6] P. SIMONART and H. VERACHTERT, *Bull. Soc. Chim. Biol.* **48**, 943 (1966).
[7] R. F. CURTIS, P. C. HARRIES, C. H. HASSALL and J. D. LEVI, *Biochem. J.* **90**, 43 (1964).
[8] G. PETTERSSON, *Acta Chem. Scand.* **19**, 414 (1965).
[9] E. GAUMANN, *Phytopath.* 2, **49**, 211 (1964).
[10] J. L. HARLEY, *The Biology of Mycorrhiza*, Leonard Hill, London (1959).

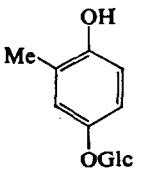
unlikely in view of its occurrence as a glucoside in the leaf, and not free in the roots, and of its very restricted distribution.

The discovery of orcinol glucoside (I) in the Ericaceae is not surprising in view of the wide range of other phenols reported in the family.[11] Indeed, with its discovery, it is clear that the family contains all three types of dihydroxybenzene. Thus, derivatives of the *p*-isomer hydroquinone (e.g. arbutin) have long been known in these plants and the *o*-isomer catechol has recently been found as the β-D-glucoside in leaves of *Gaultheria*.[12]

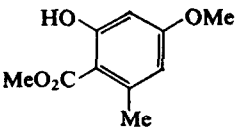
Two other close structural analogues to (I) with C-Methyl attachments have been isolated from the family, namely homoarbutin (II) from *Pyrola incarnata* roots[13] and sparassol (III) reported in *Rhododendron japonicum* roots in 1930.[14]



(I) Orcinol glucoside



(II) Homoarbutin



(III) Sparassol

The distribution of (I) in the Ericaceae is somewhat erratic but it does occur in all three sub-families studied. In the Rhododendroideae, it was found in *Phyllodoce empetriformis* (Sm.) Don, *P. caerulea* (L.) Bab. and *P. nipponica* Mak. and in *Rhododendron spiciferum* Franch, *R. decorum* Franch, *R. traillianum* Forr. S. W. W. Sm. and *R. thomsonii* Hook. In the Ericoideae, there is the single occurrence in *Erica arborea* L. var. *alpina*; it was not found in nine other *Erica* species or cultirars. Finally in the Vaccinioideae, it was found in *Pieris nana* and *P. japonica* (Thunb.) Don cv. *variegata*. From the taxonomic viewpoint, it is mainly of interest as probably characterizing the genus *Phyllodoce*, being present in all three species available of a total of seven species. That it may prove to be a useful generic character was shown by its absence from all species studied of related genera in the same sub-family, namely *Daboecia*, *Ledothamnus*, *Loiseleuria*.

EXPERIMENTAL

Plant Material

Plants of the Ericaceae were obtained from the University of Liverpool Botanic Garden and were identified by Mr. J. K. Hulme and Dr. J. Cullen; voucher specimens were deposited in the Garden Herbarium. A sample of *Erica arborea* var. *alpina* was also obtained from the University of London Supply Unit, through the courtesy of Dr. J. B. Pridham.

Detection of Orcinol

Dried leaf samples of 108 species belonging to twenty-three genera of the Ericaceae were heated in 2 N HCl for 20 min at 100°, cooled and extracted into ether. The 5% Na_2CO_3-soluble fraction of each extract was dried, concentrated and chromatographed two-dimensionally on silica gel in 10% HOAc in $CHCl_3$ and 45% EtOAc in benzene and on cellulose MN300 using benzene–MeOH–HOAc (4:1:5) and 6% HOAc. Orcinol was detected in ten species (listed in text) on chromatoplates as a dark absorbing spot under short u.v. light and as a red spot, after spraying with vanillin–HCl. Its R_f and other properties, and those of related simple phenols, are given in Table 1. Larger quantities were obtained, for identification purposes, by preparative TLC using the solvents listed above.

[11] R. Hegnauer, *Chemotaxonomie der Pflanzen*, Vol. 4, Birkhauser Verlag, Basel (1966).
[12] G. H. N. Towers, A. Tse and W. S. G. Maas, *Phytochem.* **5**, 677 (1966).
[13] H. Inouye, *J. Pharm. Soc. Japan* **78**, 298, 301 (1958).
[14] K. Kinoshita *Acta Phytochim. (Tokyo)* **5**, 157 (1930).

Isolation of Orcinol β-D-Glucoside

A 70% EtOH extract of *E. arborea* var. *alpina* leaf was concentrated and subjected to electrophoresis on Whatman No. 3 paper at 300 mV in 0·1 M borate buffer, pH 8·8, for 3 hr. A dark-blue band, absorbing in the short u.v., was found to move towards the anode (*ca.* 2 cm), while all the other phenolic constituents, visible in long-wavelength u.v., moved towards the cathode. The band was purified by TLC on cellulose and then had the properties shown in Table 1. Acid hydrolysis (20 min, N HCl 100°) or enzymic hydrolysis (β-glucosidase, 1 hr 37°) gave orcinol and glucose.

Orcinol D-glucoside was obtained by feeding orcinol (0·1% aq. soln) to germinating *Vicia faba* seeds for 3 days in the dark. The product was purified by paper chromatography and was identical in every way (Table 1) with natural material from *Erica*. Resorcinol β-D-glucoside was obtained similarly and shown to differ from the *Erica* glucoside (see Table 1).

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