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PHENOLIC GLUCOSIDE-6′-SULFATE FORMATION IN *VICIA FABA*

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Abstract—The fate of hydroquinone and catechol administered to a variety of higher plants over a period of several days has been investigated. All plants yielded monoglucosides, while a small number also yielded the gentiobiosides. Fifteen of 23 species examined yielded a phenolic glucoside derivative which appeared to be identical to a compound described earlier by Pridham and Saltmarsh as a phenolic glucoside-6′-sulfate. Definitive chemical synthesis of arbutin-6′-sulfate was achieved and direct comparison with the natural derivative clearly excluded the possibility of a sulfated derivative.

INTRODUCTION

IT HAS been clearly demonstrated that when simple phenols are introduced into higher plant tissues the corresponding mono-β-D-glucopyranosides are formed as the immediate products.[1–5] The pioneering and extensive studies of Miller[6] showed that phenols and alcohols could be conjugated to form glucosides, gentiobiosides, and, on one occasion, a primeveroside, when administered over a period of several weeks. Pridham and Saltmarsh[7] observed that when seeds of *Vicia faba* were germinated in dilute solutions of phenols, the corresponding glucosides were accompanied by small quantities of a compound which they suggested to be the glucoside-6′-sulfate.

Recent studies in our laboratory[8] have confirmed and extended the observations of Pridham[9] on the distribution of the phenolic glucosylation reaction in the plant kingdom. During the course of our studies variable amounts of three derivatives of hydroquinone were observed in several plants. The major derivative in each was arbutin.[8] The distribution and identity of the two minor derivatives is discussed in this paper.

RESULTS AND DISCUSSION

Table 1 lists the products identified when 5 mM aq. solutions of catechol and hydroquinone were administered separately to a variety of plants. In all cases the monoglucosides

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[2] A. HUTCHINSON, C. ROY and G. H. N. TOWERS, *Nature, Lond.* **181,** 841 (1958).

[3] T. MIWA, S. NAKAMURA and A. SHIBATA, *Koso Kagaku, Shinpojiuma* **12,** 48 (1957); *Chem. Abstr.* **52,** 1314 (1958).

[4] C. W. NYSTROM, N. E. TOLBERT and S. H. WENDER, *Plant Physiol.* **34,** 142 (1959).

[5] J. B. PRIDHAM, *Nature, Lond.* **182,** 795 (1958).

[6] L. P. MILLER, *Contrib. Boyce Thompson Inst.* **12,** 15, 25, 29, 163, 167 (1941); *ibid.* **12,** 359, 465 (1942).

[7] J. B. PRIDHAM and M. J. SALTMARSH, *Biochem. J.* **74,** 42 (1960).

[8] A. D. M. GLASS and B. A. BOHM, *Phytochem.* **9,** 2197 (1970).

[9] J. B. PRIDHAM, *Phytochem.* **3,** 493 (1963).

TABLE 1. RESULTS OF SURVEY FOR DERIVATIVE FORMATION

Plant	Product* GLU	ABG	GENT
Ferns and Fern Allies			
Psilotum nudum (L.) Beavois	+	+	—
Adiantum caudatum (L.) Mant.	+	+	—
Campyloneuron sp.	+	+	—
Ctenitis decomposita (R.Br.) Copel.	+	—	—
Dennstaedtia wilfordii (Moore) koidz	+	+	—
Nephrolepis exaltata (L.) Schott	+	+	—
Phlebodium aureum J. Smith	+	+	—
Pityrogramma calomelanos (L.) Link	+	+	—
Pteris cretica L.	+	+	—
Angiosperms			
Acer macrophyllum Pursh	+	+	—
Cytisus scoparius (L.) Link	+	—	—
Epilobium angustifolium L.	+	+	—
Gaultheria adenothrix (Mig.) Maxim.	+	—	—
Lycopersicum esculentum Mill.	+	trace	+
Pelargonium cv.	+	—	—
Peperomia maculosa Hook.	+	+	—
Phaseolus vulgaris L.	+	—	—
Plantago major L.	+	+	—
Rubus parviflorus Nutt.	+	—	+
Sorbus aucuparia L.	+	—	—
Tradescantia sp.	+	+	—
Trifolium pratense L.	+	+	—
Triticum vulgare Vill.	+	—	+
Vicia faba L.	+	+	trace
Zea mays L.	+	trace	—

* Key to products: GLU, glucoside; ABG, arbutigenin GENT, gentiobioside.

were formed in large concentrations. In three of 25 species examined the corresponding gentiobiosides were also observed, with trace amounts possibly being present in a fourth. The observed gentiobiosides yielded two equivalents of glucose per equivalent of phenol after acid hydrolysis and were chromatographically inseparable from authentic gentiobiosides in five solvents.

Fifteen of the remaining species had, in addition to the monoglucoside, varying quantities of an unknown phenolic glucoside derivative. Two additional species may have formed trace amounts. The unknown compound possessed the properties recorded by Pridham and Saltmarsh[7] for a suspected glucoside-6′-sulfate in *Vicia faba*: typical simple glucoside colour reaction with diazotized *p*-nitroaniline, UV spectrum identical with the simple glucoside, and extreme lability with hydrolysis under the mildest conditions giving the corresponding glucoside. Identical situations existed when either phenol was used as the test substance. Attempts were made to isolate the unknown from *V. faba*, *Pityrogramma calomelanos*, and *Psilotum nudum* in which quantities equal to the monoglucosides were found. Crystallization of a pure sample was not accomplished due to ease with which hydrolysis occurred.

The unknown (hereafter called arbutigenin) was extremely labile giving rise to arbutin spontaneously during chromatography in neutral alcoholic solvents, whilst standing in aqueous or alcoholic solution, or upon very brief (1 min) treatment with dilute acid or alkali (0·1 M). Complete hydrolysis with HCl at 100° gave hydroquinone and glucose; no other organic products were observed. Elution of arbutigenin from chromatography paper (Whatman 3MM) followed by alkaline hydrolysis (0·1 M at 100°) gave hydroquinone, glucose, and a faint precipitate with $BaCl_2$. Treatment of an equal area of equivalently treated paper also gave a faint sulfate test with $BaCl_2$; the positive sulfate test with the arbutigenin hydrolysate is thus inconclusive.

Several of the properties of the putative sulfate are incompatable with the properties of sugar sulfates discussed by Turvey.[10] Whereas arbutigenin was readily hydrolyzed by brief treatment with either acid or base, sugar sulfates are resistant to these hydrolytic conditions. Turvey[10] reported 'slow hydrolysis' of glucose-3-sulfate and galactose-4-sulfate when treated with 0·15 M HCl at 100°. Furthermore, Soda and Nagai[11] reported the relative rates of hydrolysis of sugar sulfates in acid to be 3- > 4- > 6-. In the presence of strong base sugar sulfate hydrolysis is very slow. Di-*o*-isopropylidene-D-galactose-6-sulfate was stable to 2 M NaOH at 100° for several hours.[12] With dilute base a different process obtains.[10] Under these conditions a base catalyzed elimination of sulfate occurs with concurrent production of an anhydro sugar. In the case of glucose-6-sulfate, 3,6-anhydroglucose would be formed. As stated above, hydrolysis of arbutigenin produced aglucone and glucose; other sugar derivatives were not observed.

Pridham and Saltmarsh[7] also reported that the compound from *Vicia faba* did not respond to a glucosulfatase preparation.

Pridham and Saltmarsh[7] located the substituent at the 6′-position of resorcinol glucoside by methylation according to the method of Kuhn *et al.*[13] Resorcinol monomethyl ether and 2,3,4-tri-*o*-methylglucose were obtained pointing to position-6 as the substituent locus. However, Haworth *et al.*[14] demonstrated the tendency of substituents on the glucose molecule to migrate towards the 6-position under the conditions of this methylation. The lability of glucose substituents with subsequent migration to position-6 have also been discussed by Haslam *et al.*,[15] Entlicher and Kocourek,[16] and Pearl and Darling.[17] Thus, absolute location of the substituent at position-6 cannot be made solely on the basis of the methylation experiment.

Several attempts were made in our laboratory to incorporate ^{35}S sulfate into arbutigenin. Label was administered along with hydroquinone glucoside or with hydroquinone to germinating *Vicia faba* seeds. Although arbutigenin was formed, no incorporation of radioactivity from the labelled sulfur could be detected.

We next turned to the synthesis of arbutin-6′-sulfate by a definitive route so that comparison of the product with arbutigenin could be made. The chromatographic behaviour of arbutigenin and synthetic arbutin-6′-sulfate were different. Electrophoretic analysis showed further differences. Using Whatman 3MM paper, pH 2, 3000 V, and 200 mA for 30 min arbutigenin and arbutin migrated 1 cm toward the positive pole while arbutin-6′-sulfate migrated 11 cm. Finally, arbutin-6′-sulfate was unaffected by 0·1 M alcoholic NaOH. Treatment, with boiling 0·1 M NaOH for 10 min yielded unchanged sulfate and only a

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[14] W. N. Haworth, E. L. Hirst and E. G. Teece, *J. Chem. Soc.* 2858 (1931).
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[16] G. Entlicher and J. Kocourek, *Arch. Biochem. Biophys.* **118**, 305 (1967).
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trace of a compound which gave a colour reaction identical to arbutin with diazotized *p*-nitroaniline but failed to co-chromatograph with arbutin. This is presumably hydroquinone-3,6-anhydroglucoside.

Two other possible derivatives were then considered. Arbutin-6′-acetate (pyroside) is known to occur naturally, having been isolated by Friedrich[18] from *Pyrus communis* as well as from *Vaccinium vitis-idaea*. Synthesis of this compound was achieved by the method of Entlicher and Kocourek;[16] chromatographic comparisons showed that arbutigenin was not the 6′-acetate derivative. We rejected the possibility of a phosphorylated derivative on the basis of the failure to detect inorganic phosphate following hydrolysis of arbutigenin, the lack of electrophoretic mobility of arbutigenin, and the stability of glucose-6-phosphate to the conditions which effected complete hydrolysis of arbutigenin.

A recent note by Nanayakkara *et al.*[19] describes the formation of phenolic glycoside phosphates in *Vicia faba* and *Pisum sativum*. Results leading to this conclusion (J. B. Pridham, personal communication) are convincing. The extreme lability of arbutigenin in our hands as well as its failure to migrate in an electric field suggest a compound different from that observed by Pridham and his coworkers. Our repeated efforts to isolate workable amounts of pure arbutigenin have failed. We are thus unable to suggest an alternative structure but we are convinced that the compound is not a sulfate. Preliminary observations do not support the existence of a phosphate derivative but this possibility deserves further examination.

TABLE 2. CHROMATOGRAPHIC PROPERTIES OF PHENOLIC DERIVATIVES

Compound	Solvents				
	A	B	C	D	E
Catechol glucoside	90*	66	80	85	83
Catechol gentiobioside	93	49	61	67	31
Catechol glucoside-6′-sulphate	88	58	64	78	32
Hydroquinone glucoside	89	52	78	66	63
Hydroquinone gentiobioside	92	35	51	46	23
Hydroquinone glucoside-6′-sulphate	87	24	54	60	24
Arbutigenin	90		45		63

Electrophoresis

Compound	Migration
Arbutigenin	1 cm
Hydroquinone glucoside	1 cm
Hydroquinone glucoside-6′-sulphate	11 cm

* Chromatographic data for the above sulphated compounds and arbutigenin were determined on Whatman No. 1 paper. All other data were determined on 0·5 mm Avicel microcrystalline cellulose plates. All R_fs have been multiplied by 100.

Key to solvents: A, 2% formic acid: B, *n*-BuOH–EtOH–H_2O (40:11:19); C, *n*-BuOH–pyridine–H_2O (6:4:3); D, 80% *iso*PrOH; E, *t*-BuOH–HOAc–H_2O (3:1:1).

Electrophoretograms were developed at pH 2, with an applied voltage of 3000 V, and an initial current of 200 mA. Electrophoretograms were developed for 30 min using Whatman No. 3 paper.

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EXPERIMENTAL

Survey of plants. Solutions of either catechol or hydroquinone (5 mM) were administered to the listed plants (Table 1) through cut petioles. After 5 days metabolism the plant material was extracted with boiling 80% EtOH. Evaporation of the solvent yielded a residue from which the water soluble fraction was removed with the aid of Celite. This aq. extract, after concentration, was chromatographed on Whatman No. 1 paper using *t*-BuOH–HOAc–H_2O (3:1:1) for the first direction and *n*-BuOH–pyridine–H_2O (6:4:3) for the second. The products were visualized by means of diazotized *p*-nitroaniline followed by a 5% NaOH overspray. Other solvents used for comparison studies are listed in Table 2.

Synthesis of standard compounds. The gentiobiosides of catechol and hydroquinone were prepared according to the procedure of Helferich and Schmitz-Hillebrecht.[20] The products were purified by chromatography on Whatman No. 3MM paper using the solvents described above (and see Table 2).

Arbutin-6′-sulfate was prepared by a modification of the method employed by Haslam[15] to synthesize arbutin-6′-acetate 6′-*O*-Triphenylmethyl-2′,3′,4′,4-tetra-*O*-acetylarbutin was synthesized according to Haslam[15] (Obs m.p. 197°; Lit. m.p. 197–198°). Removal of the trityl group with HBr in HOAc yielded 2′,3′,4′,4-tetra-*o*-acetylarbutin (Obs. m.p. 147–148°; Lit. m.p. 147–148°). Sulfation of the vacant 6-hydroxyl was accomplished with H_2SO_4, and dicyclohexylcarbodiimide in dimethylformamide according to the procedure of Hoiberg and Mumma.[21] Removal of the protecting acetyl groups was performed using anhydrous ammonia in methanol and the product was purified by chromatography. Arbutin-6′-acetate was prepared according to the method of Entlicher and Kocourek.[16]

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[21] C. P. Hoiberg and R. O. Mumma, *J. Am. Chem. Soc.* **91**, 15 (1969).

Key Word Index—*Vicia faba*; Leguminosae; ferns; angiosperms; phenolic glucoside-6′-sulfate; catechol; hydroquinone; gentiobioside.