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THE PHENOL GLUCOSYLATION REACTION IN THE PLANT KINGDOM

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Abstract—The ability of various plant tissues to convert quinol and resorcinol to the corresponding mono-β-D-glucopyranosides *in vivo* has been studied. This activity is prominent in the angiosperms (except for some water plants) and gymnosperms and appears to be entirely absent in the algae and fungi. The significance of these findings is discussed.

INTRODUCTION

SEVERAL workers have clearly demonstrated that foreign phenols when introduced into the leaves and seedlings of higher flowering plants are rapidly converted to mono-β-D-glucopyranoside derivatives.[1-9] The D-glucose donor for these reactions *in vivo* is almost certainly a nucleoside pyrophosphate derivative, probably uridine diphosphoglucose (UDPG), the reaction proceeding as follows:

$$\text{UDPG} + \text{Ar. OH} \xrightarrow{\text{UDPG: phenol glucosyltransferase}} \text{Ar. OG} + \text{UDP}$$

Evidence in favour of such a reaction occurring *in vivo* is:

(1) glucosylation of phenols can be effected *in vitro* using UDPG and plant extracts;[7,10-12]
(2) UDPG would appear to be ubiquitous in higher plants;[13]
(3) a reaction involving a 'high energy' glucose donor would be energetically favourable. All attempts to carry out enzymic synthesis of *O*-β-D-glucopyranosides of phenols using "low energy" glucose donors (e.g. α-D-glucose-1-phosphate or glucose disaccharides) and plant enzymes have, so far, failed.[14]

[1] G. CIAMICIAN and C. RAVENNA, *Atti reale accad. Lincei*, **25**, 3 (1916).
[2] T. MIWA, S. NAKAMURA and A. SHIBATA, *Kôso Kagaku Shinpojiuma*, **12**, 48 (1957); *Chem. Abstracts*, **52**, 1314 (1958).
[3] A. HUTCHINSON, C. ROY and G. H. N. TOWERS, *Nature*, **181**, 841 (1958).
[4] J. B. PRIDHAM, *Nature*, **182**, 795 (1958).
[5] C. W. NYSTROM, N. E. TOLBERT and S. H. WENDER, *Plant Physiol.* **34**, 142 (1959).
[6] T. KOSUGE and E. E. CONN, *J. Biol. Chem.* **234**, 2133 (1959).
[7] J. B. PRIDHAM and M. J. SALTMARSH, *Biochem. J.* **74**, 42P (1960); *Biochem. J.* **87**, 218 (1963).
[8] J. B. HARBORNE and J. J. CORNER, *Biochem. J.* **81**, 242 (1961).
[9] V. C. RUNECKLES and K. WOOLRICH, *Phytochemistry*, **2**, 1 (1963).
[10] T. YAMAHA and C. E. CARDINI, *Biochem. Biophys. Acta*, **86**, 127 (1960).
[11] G. J. DUTTON and A. M. DUNCAN, *Biochem. J.* **77**, 18P (1960).
[12] G. A. BARBER, *Biochem.* **1**, 463 (1962).
[13] Occurrence of UDPG briefly reviewed in ref. 7.
[14] C. E. CARDINI and T. YAMAHA, *Nature*, **182**, 1446 (1958). J. B. PRIDHAM, *Biochem. J.* **76**, 13 (1960). J. D. ANDERSON, L. HOUGH and J. B. PRIDHAM, *Biochem. J.* **77**, 564 (1960).

The following investigation was mainly undertaken in order to discover whether lower plants could glucosylate phenolic hydroxyl groups and also to survey the occurrence of this activity in gymnosperms and a wide variety of angiosperms.

RESULTS AND DISCUSSION

Table 1 shows clearly that the ability to glucosylate phenols is possessed by most angiosperms and that it is not confined to leaves and developing seedlings. Within this group,

TABLE 1. FORMATION OF β-D-GLUCOPYRANOSIDES OF QUINOL AND RESORCINOL BY PLANTS

Plant	Organ or tissue tested	Glucoside formed from*	
		Quinol	Resorcinol
Angiosperms			
Arrhenatherum elatius (L.) Beauv. ex J. & C. Presl.	leaf		+
Castanea sativa Mill.	leaf	+ +	+ +
Citrus sirensis Osbeck	leaf	+ +	+ +
Cotyledon paraguayense E. Walther	stem slices	+ +	+ +
Cotyledon sp.	leaf slices	+	+
Darlingtonia californica Torr.	leaf		+ +
Deschampsia flexuosa (L.) Trin.	leaf	+ +	+ +
Drosera capensis L.	leaf	+	+
Elodea canadensis Michx.	whole organism	−	−
Ficus elastica Roxb.	leaf	+ +	+ +
Ilex aquifolium L.	leaf	+ +	+ +
Iris germanica L.	leaf	+ +	+ +
Lemna minor L.	whole organism	−	−
Narcissus jonquilla L.	leaf	+	+
Nepenthes hookeriana Lindl.	leaf	+ +	+ +
Nymphaea alba L.	shoot	+ +	+ +
Paphiopedilum insigne Pfitz.	leaf	+ +	+ +
Polygonatum multiflorum (L.) All.	rhizome	+	+ +
Quercus robur L.	leaf	+ +	+ +
Solanum tuberosum L.	tuber slices	+	+
Taraxacum officinale Weber	leaf	+ +	+ +
	floret	+	+
	tap root	+	+
Utricularia vulgaris L.	whole organism	−	−
Viscum album L.	leaf	+ +	+ +
Gymnosperms			
Cycas revoluta Thunb.	leaf	+ +	+ +
Cupressus lawsoniana A. Murray	leaf	+ +	+ +
Ginkgo biloba L.	leaf	+ +	+ +
Larix (Mill.) sp.	leaf	+ +	+ +
Pinus radiata D. Don	leaf	+ +	+ +
Ferns			
Equisitum arvense L.	branch	+ +	+ +
Polypodium (L.) sp.	leaf	+	+
Polystichum setiferum (Forsk.) Woynar	leaf	+	+
Mosses			
Acrocladium cuspidatum (Hedw.) Lindb.	shoot	±	±
Atrichum undulatum (Hedw.) P. Beauv.	shoot	+	+
Brachythecium rutabulum (Hedw.) B. & S.	shoot	−	−
Cratoneuron commutatum (Hedw.) Roth.	shoot	−	−
Ctenidium molluscum (Hedw.) Mitt.	shoot	±	±

TABLE 1 (continued)

Plant	Organ or tissue tested	Glucoside formed from*	
		Quinol	Resorcinol
Drepanocladus fluitans (Hedw.) warnst.	shoot	±	±
Funaria hygrometrica Hedw.	shoot		+
Leucobryum glaucum (Hedw.) Schp.	shoot	±	±
Philonatis fontana (Hedw.) Brid.	shoot	+	+
Pseudoscleropodium purum (Hedw.) Fleisch.	shoot	−	−
Sphagnum papillosum Lindb.	shoot	±	±
Liverwort			
Marchantia polymorpha L.	thallus	+	+
Algae			
Ascophyllum nodosum Le Jol.	thallus	−	−
Dilsea edulis Stackhouse	thallus	−	−
Fucus serratus L.	thallus	−	−
Fucus vesiculosus L.	thallus	−	−
Gigatina stellata Batt.	thallus	−	−
Halidrys siliquosa Lyngb.	thallus	−	−
Laminaria digitata Lamouroux	thallus	−	−
Pelvetia caniculata Decaisne & Thuret	thallus	−	−
Ulva lactuca L.	thallus	−	−
Uronema gigas Vischer	whole cells	−	−
Fungi			
Aspergillus niger van Tiegh.	mycelium	−	−
Psalliota campestris (Fr.) Quel.	fruiting body	−	−

*−, no glucoside detected; ±, traces present; +, small amounts; ++, high concentrations formed.

plants giving a positive reaction included monocotyledenous and both woody and herbaceous dicotyledenous species some of which are succulents or parasites. The results with three species of water plants, however, were unexpectedly negative. Neither *Utricularia vulgaris* nor *Lemna minor* could under any circumstances be induced to form glucosides; with *Elodea canadensis* glucosylation was doubtful, although traces may have been formed when resorcinol was fed in 0·25% concentration. Attempts to synthesize *m*-hydroxyphenyl-β-D-glucopyranoside using an enzyme preparation from *Lemna* with UDPG and resorcinol also failed.

All the gymnosperms that were tested also converted quinol and resorcinol to the corresponding mono-β-D-glucosides, and the activities exhibited by the leaves of these species appeared to be as high as those of the angiosperms. The species of ferns that were examined were also able to form glucosides but, in general, the activity appeared to be somewhat lower than that of the flowering plants. Some activity was present in most of the mosses that were tested but was extremely low in the majority of cases. Incubating an acetone powder prepared from *Cratoneuron commutatum* with UDPG and resorcinol produced no glucoside.

No glucosylation reactions were observed with fungi or with algae from fresh or salt water habitats, but the liverwort, *Marchantia polymorpha*, did exhibit definite activity. These latter results confirm the findings of Roy[15] who was unable to demonstrate the formation of phlorin (3,5-dihydroxyphenyl-β-D-glucopyranoside) when species of *Ulva*, *Fucus*, *Laminaria*, *Agardhiella* and *Polysiphonia* were treated with phloroglucinol and ^{14}C-labelled glucose. *Marchantia* produced a small amount of phlorin under similar conditions (cf.[3]).

It would appear from the results in Table 1 that a marked ability to glucosylate phenols is

[15] C. ROY, M.Sc. Thesis, McGill University, Montreal, Canada (1959).

characteristic of the majority of higher plants, but that this reaction is absent or occurs at a very slow rate in Bryophytes and Thallophytes. The failure of a particular species to glucosylate quinol or resorcinol can presumably be accounted for by the absence of the necessary enzyme and/or the glucose donor. There is every reason to suppose that nucleoside diphosphoglucose derivatives are common constituents of all plant life and therefore the inability to form glucosides is probably due to an enzyme deficiency. This is certainly borne out by the present *in-vitro* experiments with acetone powders from *Lemna* and *Cratoneuron*. Other factors which could interfere with studies of this kind using whole organisms or plant organs are: (1) poor uptake of the phenol by the tissue and (2) inhibition of the enzyme system by the phenol. In this particular study no difficulties in uptake were apparent, and where species exhibited an initial negative activity, the experiment was repeated using lower concentrations of the phenol in order to try and obviate possible toxic effects.

If, as has often been suggested, glucosylation serves as one method for the detoxification of harmful phenolic compounds which could either arise from normal plant metabolism or from the environment,[16] it would be interesting to know if the lower forms of plant life have other methods for dealing with these compounds. For example, this study with species of *Aspergillus* and *Psalliota* and investigations by Towers[17] and Roy[15] with *Aspergillus giganteus* Wehmer and species of *Penicillium* and *Rhizopus* suggest that fungi are unable to transfer glucosyl residues to phenols *in vivo*, and it is probable that detoxification of these compounds by fungi occurs by oxidative processes.[18] On the other hand, can the seaweeds, which are rich in sulphated polysaccharides, detoxify phenols by a sulphation process similar to that occurring in animal tissues?[19]

Reports of phenolic glucosides or other glycosides occurring naturally in many of the lower groups of plants are very few in number although it must be stressed that detailed investigations of these plants has not so far been carried out. It would be also interesting to know whether the activity of the enzyme system responsible for the glucosylation of quinol and resorcinol is related to the concentration of naturally occurring phenolic glucosides in the tissue. It should not necessarily be assumed that such a relationship exists, since enzymes specific for the glucosylation of phenols other than quinol and resorcinol may be present.

It would also be useful to investigate further examples of fresh-water angiosperms and algae. In the former group only the free-floating plants were inactive; *Nymphaea*, which has a root, showed a normal angiosperm activity. Further comparative work along these lines might yield results of taxonomic interest.

MATERIALS AND METHODS

Plant specimens were obtained from the Botany Department and grounds of Royal Holloway College and the University of London Botanical Supply Unit.

Feeding experiments were carried out at room temperature for periods of 12–24 hr using 1% aqueous solutions of quinol and resorcinol. The cut ends of the various plant organs that were examined were placed in the phenol solutions. *Elodea*, *Utricularia*, the algae and all tissue slices were completely immersed in aerated solutions and *Lemna* was allowed to float on the surface of solutions. In all cases control experiments, replacing phenol solutions with

[16] Reviewed by J. B. PRIDHAM in *Phenolics in Plants in Health and Disease* (Ed. J. B. PRIDHAM), p. 9, Pergamon Press, London (1960).
[17] G. H. N. TOWERS, unpublished results.
[18] D. WOODCOCK in ref. 16, p. 75.
[19] R. T. WILLIAMS, *Detoxication Mechanisms*, Wiley, New York (1947).

water, were carried out. After the required feeding period the tissues were carefully washed with water, blotted dry, and then macerated with hot 70% aqueous methanol. The extracts were filtered, concentrated under reduced pressure at 40° and then examined on paper chromatograms (butan-1-ol–ethanol–water 40:11:19, by volume; diazotized *p*-nitroaniline/NaOH spray reagent[20]) together with authentic specimens of arbutin (*p*-hydroxyphenyl-β-D-glucopyranoside) and *m*-hydroxyphenyl-β-D-glucopyranoside. These standard compounds give characteristic, stable azo dyes with the diazonium reagent which under alkaline conditions are blue and red, respectively. Species which gave negative results under these conditions were re-examined using lower phenol concentrations (0·5 and 0·25% w/v). Penetration of the tissues by the phenols was in all cases indicated by the presence of relatively large amounts of the free compounds in the methanolic extracts.

Acetone powders of *Lemna* and *Cratoneuron* were prepared and extracted, separately, with 0·05 M-Tris-HCl buffer (pH 7·2) containing 0·5% L-cysteine (ca. 1 g acetone powder to 50 ml buffer). These extracts were then incubated at 35° with resorcinol and UDPG using the conditions described by Pridham and Saltmarsh.[7] Control digests omitting the UDPG were also set up. Paper chromatographic examination, using the solvent and spray reagent given above, showed that no *m*-hydroxyphenyl-β-D-glucopyranoside was formed in either case.

Acknowledgements—The author is indebted to Drs. J. Evans and E. Lodge for helpful discussions.

[20] T. SWAIN, *Biochem. J.* 53, 200 (1953).