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SHORT COMMUNICATION

DHURRIN SYNTHESIS IN EXCISED SHOOTS AND ROOTS OF SORGHUM SEEDLINGS*

P. F. REAY† and ERIC E. CONN

Department of Biochemistry and Biophysics, University of California, Davis, California 95616, U.S.A

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Abstract—Roots and shoots excised from sorghum seedlings incorporated the label of *p*-hydroxymandelonitrile-1-^{14}C but not the label of *p*-hydroxybenzaldehyde-7-^{14}C into the cyanogenic glucoside, dhurrin. Tyrosine-U-^{14}C significantly labelled the glucoside only in shoots. Both tissues glucosylated *p*-hydroxybenzaldehyde-7-^{14}C to form *p*-glucosyloxybenzaldehyde.

INTRODUCTION

CYANOGENIC glucosides are reported to be formed from α-hydroxynitriles in cherry laurel leaves and in flax seedlings.[1] In flax, neither acetone nor HCN, derivable from the α-hydroxynitrile was found to be incorporated into the glucoside, linamarin.[2,3] However, Koukol *et al.*[4] had reported that *p*-hydroxybenzaldehyde was a precursor of dhurrin, the cyanogenic glucoside of sorghum, although they found that the seedlings failed to incorporate label from HCN-^{14}C into the glucoside.

Since the apparent incorporation of an aldehyde into dhurrin did not involve addition to HCN, and thus involve an α-hydroxynitrile, its importance in the biosynthesis of dhurrin was investigated by feeding *p*-hydroxymandelonitrile to sorghum tissues. Since it seemed likely that *p*-hydroxybenzaldehyde was in fact incorporated into a different glycoside,[5] the identity of this glycoside was re-examined.

RESULTS AND DISCUSSION

As shown in Table 1, excised shoots extensively incorporated tyrosine-U-^{14}C into dhurrin with 49% of the ^{14}C taken up by the shoots appearing in the cyanogenic glucoside. Because of the small amount (1 mμmole) of tyrosine-U-^{14}C administered, this corresponded to a dilution factor of 36,800. In contrast to an earlier report[4] it was possible to isolate a small amount of dhurrin from roots of sorghum seedlings. However, the isolated dhurrin had a high dilution factor and the roots did not form dhurrin from tyrosine-U-^{14}C to any significant extent.

Both the shoots and roots incorporated the label of *p*-hydroxymandelonitirile-1-^{14}C into dhurrin as shown by the extent of incorporation and the low dilution factor. This suggests that the observed incorporation of this compound by shoots is not necessarily related to the

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† Present address: Applied Biochemistry Division, D.S.I.R., Palmerston North, New Zealand.

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Table 1. Conversion of various compounds into dhurrin by roots and shoots excised from etiolated sorghum seedlings

Compound administered		Dhurrin purified (μmole)	Dhurrin %^{14}C Incorporated	Dhurrin Dilution factor	%^{14}C As cyanide Found	%^{14}C As cyanide Expected	% Recovered in hydrolysis
Tyrosine-U-^{14}C	Shoots	4·3	49*	36,800	12·4	12·5	95
(442 mμc in 1·90 μmole)	Roots	0·138	0·008*	214,000	14·1	12·5	102
p-Hydroxymandelonitrile-1-	Shoots	3·9	7·3	68	97	100	103
^{14}C (394 mμc in 5·0 μmole)	Roots	0·30	14·3	9·5	68	100	102
p-Hydroxybenzaldehyde-7-	Shoots	1·4	<0·00037†	>18,700	—		100
^{14}C (481 mμc in 2·8 μmole)	Roots	0·062	<0·00042†	>840	—		99

* Corrected for the loss of one carbon atom.
† Incorporation in the aglycone moiety only.

normal biosynthesis of dhurrin. Such a result would be obtained if a relatively non-specific *trans*-glucosylase were present and active in the shoot tissue.

Although *p*-hydroxybenzaldehyde was earlier reported to be incorporated into dhurrin,[4] neither shoots nor roots showed any significant incorporation in the experiments reported here. These results can be reconciled since it is now known that *p*-glucosyloxybenzaldehyde is separated only slightly from dhurrin by the solvents used by Koukol *et al.*[4]

p-Hydroxybenzaldehyde-7-^{14}C was incorporated moderately effectively into *p*-glucosyloxybenzaldehyde with a dilution factor of about one (Table 2). This dilution factor of unity

Table 2. Conversion of *p*-hydroxybenzaldehyde into *p*-glucosyloxybenzaldehyde by roots and shoots excised from sorghum seedlings

Compound administered		% Uptake	*p*-Glucosyloxybenzaldehyde %^{14}C	*p*-Glucosyloxybenzaldehyde Dilution factor
p-Hydroxybenzaldehyde-7-^{14}C (481 mμc in 2·8 μmole)	Shoots	94	52	0·97
	Roots	98	5	1·11

strongly suggests that *p*-hydroxybenzaldehyde and *p*-glucosyloxybenzaldehyde do not occur naturally in sorghum. At least two other probable glucosides were also found to be labelled by *p*-hydroxybenzaldehyde-7-^{14}C.

The root and shoot of sorghum were able to glucosylate both phenolic and cyanohydrin alcohol groups. This formation of phenolic glucosides is considered to be a common feature of plant tissues.[6] Nystrom *et al.*[7] reported that barley and wheat leaves form β- phenylglucoside from phenol while Pridham and Saltmarsh[6] found that corn and bean seedlings glucosylated a variety of di- and triphenols to form β-monoglucosides.

Since the alcohol group of the α-hydroxynitrile was glucosylated equally well by the root and by the shoot, its incorporation into dhurrin by the shoot does not support its role as an

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intermediate in dhurrin biosynthesis. Isolation and purification of the enzymes involved are necessary to establish their specificity for α-hydroxynitriles and is being carried out in this laboratory.

EXPERIMENTAL

p-Hydroxymandelonitrile-1-^{14}C was synthesized after Laderberg *et al.*[8] Glucose-U-^{14}C and tyrosine-U-^{14}C were obtained from New England Nuclear, Boston, and emulsin (β-glucosidase) from Calbiochem, Los Angeles. *p*-Hydroxybenzaldehyde-7-^{14}C was a gift from Dr. A. C. Neish. *p*-Glucosyloxybenzaldehyde was a gift from Dr. J. Stoker. Dhurrin used as a chromatographic standard was isolated as described previously.[9]

Descending paper chromatography was performed on Whatman No. 1 and No. 3 MM filter paper using the following solvents: 1, 2-butanone–acetone–water (30:10:6, v/v/v); 2, *n*-BuOH–H_2O (50:9, v/v); 3, *n*-BuOH–$PrCO_2H$–H_2O (62:31:43, v/v/v); 4, EtOH–NH_4OH–H_2O (80:5:15, v/v); 5, *n*-BuOH–pyridine–H_2O (6:4:3, v/v/v); and 6, *n*-BuOH–HOAc–H_2O (12:3:5, v/v/v).

TLC was performed with the following solvents: 7, benzene–EtOAc–HCO_2H–H_2O (9:21:6:3, v/v/v/v) on microcrystalline cellulose; and 8, MeOAc–*iso*-PrOH–H_2O (18:1:1, v/v/v) on silica gel.[10] The last solvent was allowed to ascend the layer three times.

Seeds of *Sorghum vulgare*, Pers. (variety Sorghum-Sudan), were soaked overnight and germinated for 2 days in the dark. For each treatment thirty shoots (*ca.* 0·5 g) excised from the etiolated seedlings were fed the radioactive compound under illumination. Similarly, the roots from about fifty seedlings (*ca.* 0·5 g) were shaken at 30° in 2–3 ml water containing the radioactive compounds.

After 7 hr, the tissues were extracted[11] in MeOH–$CHCl_3$–HCO_2H at −80°. After storing at −20° for 24 hr the tissues were crushed and filtered out. The solution was split into two phases[11] and the lower phase rinsed with 1–2 ml water. The washings and the upper phase were evaporated to dryness at < 40° and the residue dissolved in 10% isopropanol. The lower phase was discarded after determination of the radioactivity.

Dhurrin was purified and identified by chromatography with solvents 1–3. It was located on the chromatograms by exposing part of the paper to ammonia vapors for 1 hr. The resulting aldenhyde gave an orange color with 2,4-dinitrophenylhydrazine (0·1% in 2 N HCl).

The specific activity of the purified dhurrin was determined by hydrolysis with emulsin in center-well flasks. The resulting HCN was trapped in 1 N NaOH and determined by the method of Aldridge.[12] An aliquot was counted in Triton-toluene scintillation fluid.[13] The specific acitivity of *p*-hydroxymandelonitrile was found similarly after dissociation in phosphate buffer, pH 6·2.

A glucoside resulting from the feeding of *p*-hydroxybenzaldehyde had the same R_fs in solvents 1 (0·62), 2 (0·42) and 3 (0·60) as authentic *p*-glucosyloxybenzaldehyde. After purification in these solvents this glucoside labelled from *p*-hydroxybenzaldehyde-7-^{14}C was treated with emulsin, and the radioactive product was found to have the same R_f (0·73) as *p*-hydroxybenzaldehyde in solvent 4, and the same absorption maximum (330 nm) in 0·1 N NaOH. The aglycone is therefore *p*-hydroxybenzaldehyde and the glucoside is of the β-configuration. The specific activity of the glucoside was determined from that of the aglycone using the latter's absorption at 330 nm.

To identify the sugar moiety of the glucoside, glucose-U-^{14}C was fed to roots and shoots, and the glucoside purified as before. The purified glucoside was then treated with 0·1 N NaOH for 2 hr to break down dhurrin, and re-chromatographed in solvent 1. Part of the glucoside was then treated with excess semicarbazide, as was authentic *p*-glucosyloxybenzaldehyde. The products were dissolved and chromatographed with solvent 7 on micro-crystalline cellulose; the radioactive product and that from *p*-glucosyloxybenzaldehyde had the same mobility. The remainder of the glucoside was then hydrolyzed with emulsin and the radioactive product identified as glucose from its mobility in solvents 5, 6 and 8. The isolated glucoside is thus *p*-β-*O*-glucosyloxybenzaldehyde.

Incorporation of the label from *p*-hydroxybenzaldehyde-7-^{14}C into dhurrin was determined after chromatography of the latter in solvent 1 and hydrolysis with 0·1 N NaOH. The aglycone was then chromatographed in solvent 4 and the radioactivity in the position of *p*-hydroxybenzaldehyde determined by adding 1 ml water to pieces of the chromatogram in a vial followed by 10 ml Triton–toluene scintillation fluid.[13]

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