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DEGRADATION OF [β-^{14}C]HORDENINE IN *HORDEUM VULGARE* PLANTS

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Key Word Index—*Hordeum vulgare*; Gramineae; [β-^{14}C]hordenine; metabolic degradation.

Abstract—[β-^{14}C]Hordenine is ultimately degraded by intact plants of *Hordeum vulgare* to C_6–C_1 intermediates that are incorporated into polymeric material.

It has been previously reported [1, 2] that the alkaloid hordenine is metabolized in *Hordeum vulgare* plants *ca* 30 days after germination. We have recently shown [3] that the carbon atom α to the nitrogen in hordenine is rapidly lost as CO_2 and is not incorporated into lignin.

In an attempt to determine the fate of the carbon atom β to the nitrogen, we prepared [β-^{14}C]hordenine and fed it to intact *H. vulgare* seedlings. After 26 hr the plants were harvested, the roots and the aerial parts were separated, ground, and extracted with methanol and water. The activities of extracts, residual material, and remaining feeding solution were determined (Table 1). The tabulated values indicate that the degradation of the β-carbon is negligible and that 95% of the absorbed activity is found in the extracts and in the residue.

From the methanolic extract radioactive hordenine, tyramine and *p*-hydroxybenzoic acid were isolated and fully characterized. The aqueous extract also contained hordenine and tyramine. On the other hand, radioactive lignin was degraded as reported [4] affording radioactive *p*-hydroxybenzaldehyde but inactive vanillin, while hydrolysis [5] of the lignin gave *p*-hydroxybenzoic acid.

The present results, together with previous ones [3], indicate that in intact *H. vulgare* plants hordenine is degraded to C_6–C_1 units before entering into lignin. This does not agree with the results encountered for barley cell suspension cultures [6] in which the intermediacy of C_6–C_2 units has been proposed. Hence, the isolation of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoic acid could be indicative of the sequential degradation outlined in Fig. 1. The enzymic oxidation of *p*-hydroxybenzaldehyde to *p*-hydroxybenzoic acid is known to occur in plant tissues [7].

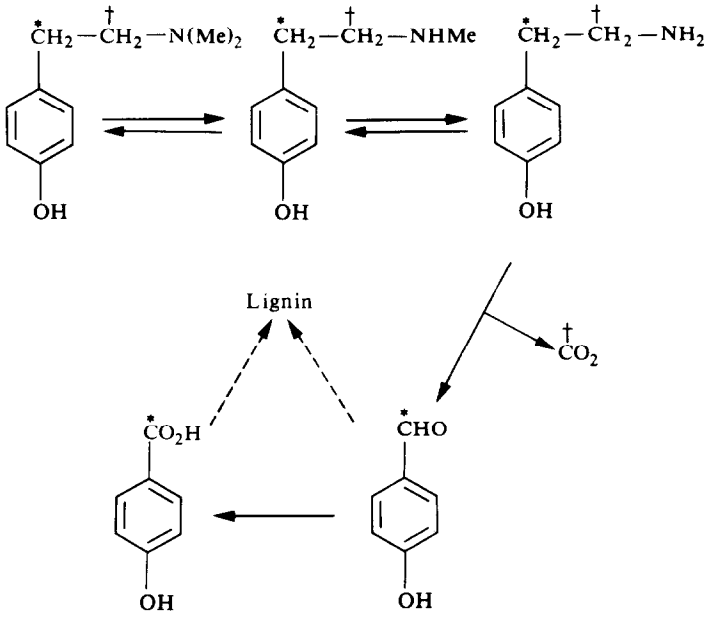

Fig. 1. Possible metabolism of hordenine in *H. vulgare* intact plants. Labelled carbon atoms are indicated by * and †.

Table 1. Feeding of [β-^{14}C]hordenine (0.183 mCi) to 400 *H. vulgare* seedlings

Fraction	Amount (g)	Sp. act. (dpm/mg)	Total act. (dpm)	Abs. incorp. (%)
Roots				
MeOH	2.28	7.76×10^4	1.77×10^8	43.4
H_2O	0.68	3.12×10^4	2.12×10^7	5.2
Residue	1.81	4.27×10^4	7.73×10^7	18.9
Aerial part				
MeOH	0.93	1.30×10^4	1.21×10^7	3.0
Residue	1.62	7.48×10^2	1.20×10^6	0.3

Radioactivity remaining in the feeding solution after 26 hr: 1.05×10^8 dpm (25.7% of the labelled hordenine).

EXPERIMENTAL

Materials and methods. The growing of plant material and feeding techniques were as previously described [3]. The counting of radioactive samples was also as used earlier. GLC was performed with a Hewlett–Packard 5830A gas chromatograph using glass columns packed with 3% OV-17 on Chromosorb W-AW-DMCS. The samples were silylated by treatment with HMDS–TMCS (99:1) for 15 min at 100°. GC/MS was performed with a Varian-Mat CH7-A mass spectrometer coupled to a Varian-Mat Data System 166 computer. Radioscanning of chromatographic paper was conducted with a Packard 7201 radioscanner. Radioactive material was obtained from the Radiochemical Centre, Amersham, U.K.

Radiochemicals. [β-^{14}C]Hordenine was synthesized by a known method [8] using a mixture of [β-^{14}C]tyramine hydrochloride (250 μCi; 50 mCi/mmol) and tyramine hydrochloride (165 mg). The labelled compound (158 mg) was purified by sublimation and its purity was controlled by TLC, IR and MS; it had a sp. act. of 190 μCi/mmol.

Feeding experiment and extraction of plant material. 400 six-day-old seedlings of *H. vulgare* (Magnif 102-INTA 1978/79) were fed with the labelled product (0.39 mg/plant). After 26 hr the seedlings were harvested and the shoots were separated from the roots. Aerial parts and roots were extracted with MeOH and the roots also with H_2O. The extracts were evapd to dryness and assayed for radioactivity (see Table 1).

Isolation and identification of extracts' components. The residue from the MeOH extract (2.28 g) was treated with petrol (2×50 ml) and the defatted residue was chromatographed through a Si gel column. This was eluted with CH_2Cl_2, CH_2Cl_2–MeOH (9:1 and 5:5), MeOH and MeOH–HCl (97:3) and monitored by TLC and radioactivity. From different fractions hordenine (33.1 mg, 4.15×10^8 dpm/mmol), tyramine (37.5 mg, 3.11×10^8 dpm/mmol) and *p*-hydroxybenzoic acid (18.0 mg, 2.21×10^8 dpm/mmol) were isolated. These compounds gave identical results (TLC, GLC, MS) to authentic standards. The TLC analysis of the residue from the aq. extract (0.68 g) indicated the presence of hordenine and tyramine. These were separated by partial sublimation at 0.5 torr and 110° for hordenine (7 mg, 2.51×10^8 dpm/mmol) and at 0.5 torr, 140–150° for tyramine (7.2 mg, 2.02×10^8 dpm/mmol).

Analysis of the insoluble residue. The radioactive residue was processed as described [9] for the isolation of lignin yielding 602 mg of product having 1.28×10^5 dpm/mg. A portion of the lignin (10 mg) was oxidized with nitrobenzene in alkaline conditions [4]. The resulting mixture was analyzed by PC showing the presence of *p*-hydroxybenzaldehyde and vanillin. Radioscanner analysis of the chromatographic paper indicated that the *p*-hydroxybenzaldehyde was active (1.52×10^8 dpm/mmol; 19.4% of total activity) whilst the vanillin was devoid of radioactivity.

Another portion of lignin (150 mg) was hydrolysed with 2 N KOH in MeOH as described [5]. The ppt formed on acidification was filtered off through cheese-cloth and the filtrate was extracted with Et_2O (3×25 ml). Evapn of the solvent afforded a ppt. that was identified (TLC, MS) as *p*-hydroxybenzoic acid (13.2 mg, 1.58×10^8 dpm/mmol).

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