Phytochemistry, Vol. 20, No. 7, pp. 1763–1764, 1981.
Printed in Great Britain.

0031-9422/81/071763-02 $02.00/0

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

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(*Received* 18 *August* 1980)

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

Abstract—Evidence is presented indicating that intact plants of *Hordeum vulgare* degrade [α-^{14}C]hordenine to $^{14}CO_2$.

In a recent paper [1] the catabolic degradation of hordenine in plant cell suspension cultures using [β-^{14}C]hordenine and [methyl-^{14}C]hordenine was reported but nothing was said about the fate of the α-carbon atom of the alkaloid. Previous work on this subject [2] indicated that hordenine is not degraded, except for the methyl groups to one-carbon fragments, because the feeding of a supposedly [α-^{14}C]hordenine led to 97% recovery of radioactivity in the methanolic and alkaline extracts. It was reported that the only radioactive compounds isolated were hordenine, *N*-methyltyramine and lignin, and that any loss as carbon dioxide was negligible.

In order to confirm the latter result, 8-day-old *Hordeum vulgare* plants were fed with [α-^{14}C]hordenine [3] and 14 days later, the plants were harvested and the roots and aerial parts were separated, dried for 36 hr, ground and successively extracted with petrol, methanol and water. The extracts were concentrated and assayed for radioactivity. The activity of the residual material and the remaining feeding solution was also determined. The results (Table 1) indicate that almost 90% of the radioactivity is lost, in contrast to that claimed previously [2].

Accordingly, we fed 6-day-old *H. vulgare* plants growing in a special chamber with [α-^{14}C]hordenine and

Table 1. Results of 14-day feeding of [α-^{14}C]hordenine to *Hordeum vulgare* intact plants

	Roots (10.10 g)			
Fraction	Amount (g)	Sp. act. (dpm/mg)	Total act. (dpm)	Absolute incorp. (%)
Petrol	0.335	1.4×10^3	4.7×10^5	0.06
MeOH	0.554	1.4×10^4	8.0×10^6	0.96
H_2O (cold)	1.400	9.4×10^3	1.3×10^7	1.58
H_2O (hot)	0.780	1.3×10^4	1.0×10^7	1.27
Residue	5.800	4.1×10^3	2.4×10^7	3.03
	Aerial parts (7.96 g)			
Fraction	Amount (g)	Sp. act. (dpm/mg)	Total act. (dpm)	Absolute incorp. (%)
Petrol	0.416	0.5×10^3	2.3×10^5	0.03
MeOH	0.980	9.6×10^3	9.4×10^6	1.13
H_2O (cold)	1.270	1.4×10^3	1.8×10^6	0.21
Residue	5.290	0.2×10^3	1.0×10^6	0.12

Radioactivity remaining in the feeding solution after 14 days: 2.5×10^7 (3.0% of the labelled hordenine).

Table 2. Formation of $^{14}CO_2$ in intact *Hordeum vulgare* plants after administration of [α-^{14}C]hordenine

Interval time (hr)	Total time (hr)	$BaCO_3$ (mg)	Sp. act. (dpm/mg)	Total act. (dpm)	Act. $BaCO_3$ / Act. hordenine × 100	Total act. recovery (%)
2	2	801.2	2.3×10^3	1.8×10^6	0.82	0.82
2	4	164.9	1.8×10^4	3.1×10^6	1.38	2.20
2	6	165.8	2.8×10^4	4.6×10^6	2.09	4.29
2	8	160.6	3.1×10^4	5.0×10^6	2.24	6.53
12.5	20.5	993.2	2.5×10^4	2.5×10^7	11.19	17.73
6	26.5	499.7	2.2×10^4	1.1×10^7	4.88	22.60
4.3	30.8	337.0	1.9×10^4	6.5×10^6	2.94	25.54
16.8	47.6	1 240.9	1.7×10^4	2.2×10^7	9.69	35.23
9	56.6	616.4	1.7×10^4	1.0×10^7	4.62	39.84
14.4	71.2	835.0	1.9×10^4	1.6×10^7	7.36	47.21
52.8	124.0	2 631.9	1.7×10^4	4.4×10^7	19.79	67.00

Radioactivity remaining in the feeding solution after 6 days: 4.5×10^7 dpm (20.3% of the labelled hordenine).

the carbon dioxide expelled by the plants was collected. A similar cold experiment was conducted in order to determine the amount of carbon dioxide eliminated under normal conditions. The results (Table 2) indicated that, taking into account the amount of alkaloid not metabolized, almost all the α-carbon atom of hordenine was eliminated as carbon dioxide. This result was complemented by the determination of the [α-^{14}C]hordenine uptake which showed that, considering the amount of alkaloid remaining in the feeding solution, almost all the radioactivity was taken up by the plants during the first 3–4 hr from the feeding (Table 3).

The present result clearly suggests that either the catabolic mechanism earlier proposed for hordenine [1, 2] has to be revised or that the biodegradation pathway of the alkaloid in plant cell suspension cultures may be different from that in intact plants.

EXPERIMENTAL

Materials and methods. Seeds of *Hordeum vulgare* (Magnif 102, INTA 78/79) were obtained from INTA, Castelar, Pcia., Buenos Aires. [α-^{14}C]Hordenine with sp. act. 1.05×10^7 dmp/mg was synthesized as already described [3]. $BaCO_3$ samples were assayed for radioactivity by the Hyamine procedure [4], and measured by scintillation counting. The seeds were immersed into 5% $Ca(ClO)_2$ solution for 1 hr, and germinated over moist sand. Seedlings were grown in a light cycle of 14 hr light and 10 hr dark. For the ensuing experiments 6-day-old seedlings were used.

Table 3. Uptake of [α-^{14}C]hordenine by 6-day-old intact barley plants

Time (hr)	Total act. (dpm)	Uptake (%)
0	3.58×10^6	0
1	1.73×10^6	52
2	1.49×10^6	59
3	1.10×10^6	70
4	9.90×10^5	73
5	1.10×10^6	70
6	9.40×10^5	74

Uptake of [α-^{14}C]*hordenine.* Seedlings were transferred to individual vials containing Vermiculite soaked in White's salt solution [5] (2 ml), and to each vial 0.1 ml of a soln of [α-^{14}C]hordenine (3.4 mg) in H_2O (2 ml) was added. At different time intervals (Table 1) the plants were removed, rinsed with water, and the remaining soln was assayed for activity.

Formation of CO_2 *from* [α-^{14}C]*hordenine.* Seedlings were transferred to glass trays containing Vermiculite imbibed in salt soln. The trays were maintained inside a special chamber having inlets for filtered air and liquids, and a gas outlet connected to a set of traps for gases. A soln of the labelled hordenine (21.1 mg) in salt soln (50 ml) was incorporated through the inlet for liquids which was then rinsed with salt soln (2 × 25 ml). The formation of CO_2, collected as $BaCO_3$, was followed for up to 6 days. The activity of the $BaCO_3$ was measured at different times (Table 2).

Incorporation of [α-^{14}C]*hordenine into* H. vulgare *plants.* Seedlings (600) were transferred to 300 vials having salt soln (1 ml); after 2 days, 0.1 ml of a soln of the labelled hordenine (118.2 mg) in H_2O (30 ml) was added to each vial. The plants were kept in the indicated lighting conditions for 14 days with periodical additions of salt soln. The plants were harvested, roots and shoots were excised, dried and ground. Both materials were continuously extracted with petrol, MeOH and H_2O for 48 hr each. The solvents were removed and the radioactivity of the respective residues was measured (Table 3).

Acknowledgements—We thank Dr. R. Mizrahi (INTA, Castelar) for the seeds and the CONICET, the SECYT and the Organization of the American States for financial support.

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