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METABOLISM OF GRAMINE IN *HORDEUM VULGARE* PLANTS: A TIME COURSE STUDY

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

Abstract—Intact *Hordeum vulgare* plants quantitatively degrade [α-^{14}C] gramine to $^{14}CO_2$.

INTRODUCTION

The simple indole alkaloid gramine, which is present in members of Gramineae [1–4], Aceraceae [5, 6], and Leguminosae [7, 8], is biosynthesized by sprouting barley (*Hordeum vulgare*) [9–11] and *Lupinus hartwegii* [12] plants from tryptophan although the intermediates in the pathway remain unknown. Gramine is produced in barley in the first days of germination and remains in the plants in detectable quantities for *ca* 30–50 days [13, 14] depending on the growing conditions. After this period no alkaloid is present in the plants although they retain part of their ability to degrade exogenous gramine.

The metabolic fate of the side-chain of gramine has been investigated in 60-day-old excised barley shoots in the dark, but in this case only 26% of the radioactivity originally located at the side-chain of the alkaloid could be recovered [15–17]. Leete [12] found that feeding labelled tryptophan to *L. hartwegii* plants produced not only labelled gramine but also labelled indole-3-carboxaldehyde, and he concluded that this compound arose from the degradation of gramine.

As part of our investigations on the metabolic degradation of alkaloids in intact *H. vulgare* plants [18], it was of considerable interest to determine the rates of absorption of gramine and conversion of its side-chain to CO_2 in intact barley plants growing under standardized conditions.

RESULTS AND DISCUSSION

Our studies were carried out in 11 21-day-old barley plants (*H. vulgare*), so that the external addition of gramine would disturb as little as possible the normal metabolic pathway of the alkaloid which is present at this stage of growth. [α-^{14}C]Gramine was fed separately to 11- and 14-day-old plants, and the radioactivity present in the expelled CO_2 measured as a function of time. The total amount of alkaloid supplied to the plants, was *ca* three-fold of the normal gramine content at that time, as determined in a separate experiment. The alkaloid absorption rate was monitored in parallel experiments under identical conditions. The corresponding kinetic profiles for gramine absorption and radioactive CO_2 evolution are presented in Figs. 1 and 2 for the 11- and 14-day-old plants respectively.

Examination of the radioactive CO_2 evolution curves revealed a few hours lag (shorter for the 14-day-old plants) followed by an increase in the overall degradation rate that reached a maximum after 60 hr for the younger plants and after 48 hr for the older plants, slowing down after this as the external supply for gramine decreased. In contrast, no lag was observed in the gramine absorption curves. The absorption rate was higher for the 14-day-old plants thus indicating a slightly more active metabolism. It is noteworthy that for the experiment starting with 11-day-old plants the maximum rate for $^{14}CO_2$ production occurred when the plants were 13–14 days old and decreased substantially during day 16, while for the experiment starting with the 14-day-old plants the maximum rate was observed when the plants were 16 days old. Hence, the lag periods were not apparently

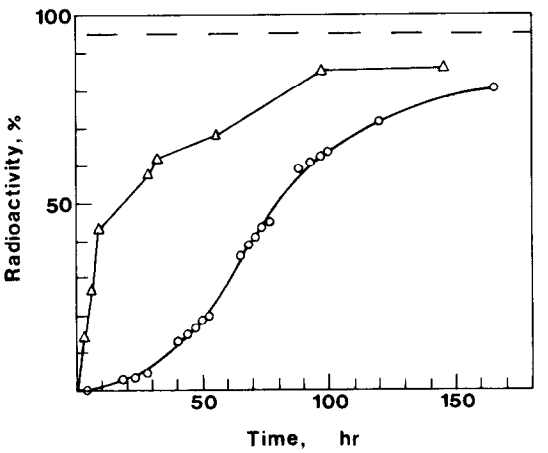

Fig. 1. Percentage of [α-^{14}C]gramine absorbed (△) and of $^{14}CO_2$ evolved (○) as a function of time by 11-day-old barley plants fed in the middle of the lighted stage. Dark periods are indicated by solid segments.

Table 1. Distribution of radioactivity in the plants 7 days after feeding [α-^{14}C]gramine to 11-day-old *H. vulgare* seedlings

	Radioactivity in MeOH–HCl extract (%)	Radioactivity in isolated gramine (%)
Roots	35	24
Aerial part	65	23

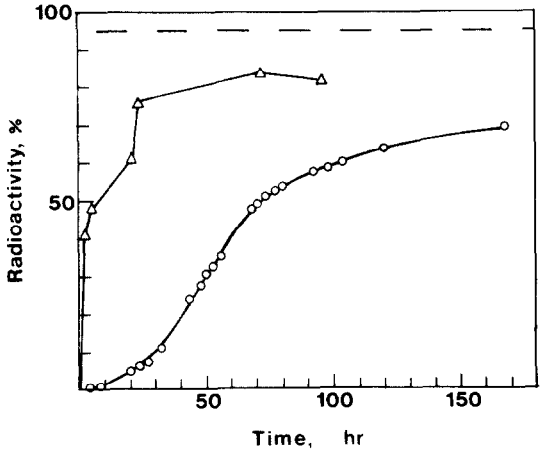

Fig. 2. Percentage of [α-^{14}C]gramine absorbed (△) and of $^{14}CO_2$ evolved (○) as a function of time by 14-day-old barley plants fed in the middle of the lighted stage. Dark periods are indicated by solid segments.

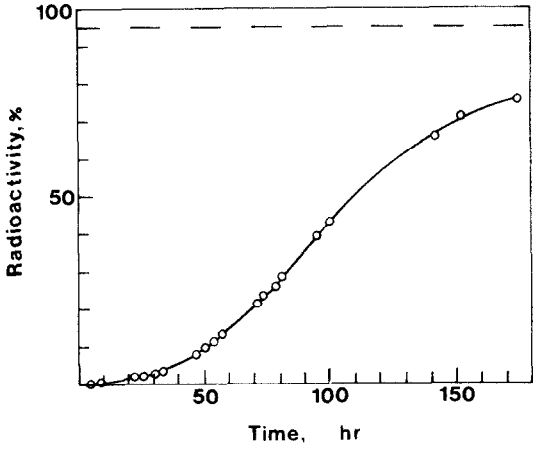

Fig. 3. Percentage of $^{14}CO_2$ evolved as a function of time by 11-day-old barley plants fed with [α-^{14}C]gramine at the beginning of the dark stage. Dark periods are indicated by solid segments.

due to the lack of enzymic activity, but to a plausible accumulation of intermediates.

In these experiments, a 95% overall conversion (97% taking into account the gramine recovered from the plants) of the side-chain of the absorbed gramine into CO_2 was obtained in a 7-day period (85% with the 14-day-old plants). The absorbed radioactivity, not evolved as $^{14}CO_2$ after this period, was found to be in the MeOH–HCl extract of the plants distributed as indicated in Table 1.

When the alkaloid was fed to 11-day-old plants during the dark stage, using barley plants grown under reversed light–dark periods, the lag in $^{14}CO_2$ production was more pronounced, reaching the maximum rate after *ca* 80 hr (Fig. 3) although the overall conversion was still 90% after 7 days.

Comparison of the alkaloid absorption curves in Figs. 1 and 2 suggests that the absorption of gramine through the roots is influenced not only by the endogenous alkaloid level, which is approximately the same in 11- and 14-day-old plants as determined in separate experiments, but also by the levels of intermediates in the degradation pathway. Our results also indicate that the α-carbon of the side-chain of gramine is quantitatively converted into CO_2 in intact growing barley plants and no evidence has been found for alternate pathways. Work is in progress to isolate and identify the intermediates present as well as to determine their effect, if any, on the absorption rate of gramine.

EXPERIMENTAL

Plants and radiochemicals. Seeds of *H. vulgare* (Magnif 102, INTA 78/79) were provided by INTA Castelar and sterilized by immersion in 5% $Ca(ClO)_2$ soln for 1 hr, and germinated in plastic trays over sand in a growth chamber equipped with Sylvania Gro Lux fluorescent lamps, and forced air circulation. The temp. inside the chamber was kept constant at 25° (23° at soil level) with 12-hr photoperiods.

[α-^{14}C]Gramine (5.8×10^6 dpm/mg) was prepared from [^{14}C]paraformaldehyde, indole, and dimethylamine by a modification of the procedure of ref. [19], and purified by column chromatography on Si gel-60 (Merck).

Radioassays. Soluble samples were dissolved in 15 ml of a dioxane soln containing naphthalene (100 g), 2,5-diphenyloxazole (7 g), 4,4'-dimethyl-2,2'-phenylene-bis-(5-phenyloxazole) (0.3 g) and H_2O (71.4 ml) in dioxane (1 l.), and assayed by scintillation counting. $Ba^{14}CO_3$ samples were suspended in 15 ml of the dioxane soln by sonication in the presence of Cab-O-Sil (Cabot, Inc.) (200 mg). Counting efficiency was 78% as calc. with standard $Ba^{14}CO_3$ samples of known activity radioassayed under similar conditions.

Formation of $^{14}CO_2$ from [α-^{14}C]gramine. 50 six-day-old plants were transferred from the germination trays to a glass container with an airtight cover fitted with air inlet and outlet tubes containing 250 g sand. Air was continuously pumped through the container by a diaphragm pump (500 ml/min) and the complete system placed in the growth chamber. At the appropriate time (11- or 14-day-old plants), [α-^{14}C]gramine (890 μg) dissolved in the minimum amount

of MeOH (*ca* 50 μl) and H_2O (10 ml) was added to the plants (*ca* 17 μg/plant), and the outlet tube was connected to a train of four $Ba(OH)_2$ traps. At suitable intervals the traps were changed and the $BaCO_3$ was filtered, washed, dried *in vacuo* and radioassayed as already described. The exact amount of radioactivity supplied to the plants was determined by counting a 50-μl aliquot from the feeding soln. At the end of each expt the sand was washed with MeOH–conc HCl (10:0.12 ml), the washings were concd *in vacuo*, dil. with H_2O, adjusted to pH 9 with 10% NaOH soln and repeatedly extracted with $CHCl_3$. The residue obtained after evapn of the solvent was mixed with cold gramine and subjected to prep. TLC on Si gel ($CHCl_3$–MeOH–NH_3, 8:2:0.1). All the radioactivity in the sand crude extract was present in the isolated gramine. From the plants, roots and aerial parts were separated and both fractions were extracted with MeOH–conc HCl. The extracts were processed as above and the results are indicated in Table 1.

Uptake of [α-^{14}C]*gramine.* 100 six-day-old seedlings were transferred to 50-ml beakers containing 10 g of sand each. At the appropriate time (11 14-day-old plants) [α-^{14}C]gramine (33.6 μg) in water (8 μl) was added to each beaker (*ca* 17 μg/plant). At suitable intervals the plants were removed and the sand was extracted with MeOH–conc HCl. After processing as described above the isolated gramine was assayed for radioactivity. Recovery of radioactive gramine from the sand was 90% as determined with appropriate controls.

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