Ornithine Decarboxylase, Polyamines, and Pyrrolizidine Alkaloids in *Senecio* and *Crotalaria*

Received for publication July 2, 1987 and in revised form October 2, 1987

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**ABSTRACT**

When tested for ornithine and arginine decarboxylases, pyrrolizidine alkaloid-bearing *Senecio riddellii*, *S. longilobus* (Compositae), and *Crotalaria retusa* (Leguminosae) plants exhibited only ornithine decarboxylase activity. This contrasts with previous studies of four species of pyrrolizidine alkaloid-bearing *Heliotropium* (Boraginaceae) in which arginine decarboxylase activity was very high relative to that of ornithine decarboxylase. Unlike *Heliotropium angiospernum* and *Heliotropium indicum*, in which endogenous arginine was the only detectable precursor of putrescine channeled into pyrrolizidines, in the species studied here—using difluoromethylornithine and difluoromethylarginine as the enzyme inhibitors—endogenous ornithine was the main if not the only precursor of putrescine converted into the alkaloid aminoaicoel moiety. In *S. riddellii* and *C. retusa* at flowering, ornithine decarboxylase activity was present mainly in leaves, especially the young ones. However, other very young organs such as inflorescence and growing roots exhibited much lower or very low activities; the enzyme activity in stems was negligible. There was no correlation between the enzyme activity and polamine or alkaloid content in either species. In both species only free polamines were detected except for *C. retusa* roots and inflorescence—with relatively very high levels of these compounds—in which conjugated putrescine, spermidine, and spermine were also found; agmatine was not identified by HPLC in any plant organ except for *C. retusa* roots with rhizobial nodules. Organ- or age-dependent differences in the polamine levels were small or insignificant. The highest alkaloid contents were found in young leaves and inflorescence.

Our previous study on PA*-bearing *Heliotropium angiospernum* and *H. indicum* (Boraginaceae) plants exposed to 14C-labeled CO2 proved that endogenous Put formed from an endogenous source is the precursor of the alkaloid aminoaicoel moiety. This confirms the biosynthetic pathway postulated from studies with exogenous Put or its possible precursors such as Orn, Arg, Spd, or Spm introduced into *Senecio* plants, as discussed in Birecka et al. (5). The effects of DFMO and DFMA, specific enzyme-activated irreversible inhibitors of ODC and ADC, respectively, on nectar radioactivity in the treated plants showed that endogenous Arg is the only detectable precursor of Put channeled into the nectines of the two borages that exhibited not only ADC but also—although low—ODC activity. However, the same borages were able to decarboxylate exogenous Orn to Put that in turn was converted into nectines. Thus, in vivo transformation of an exogenous putative precursor does not always indicate the compound formed *in situ* that normally serves as a precursor. This is especially true in the case of Put, which can derive not only from Orn but also from Arg via Agm, the product of Arg decarboxylation by ADC. Our attempt to identify the *in situ* precursor(s) of the Put channeled into pyrrolizidines in *Senecio vulgaris* were unsuccessful due to the extremely low PA content of the shoots.

We report here the effects of DFMO and DFMA on necine biosynthesis in *S. riddellii*, *S. longilobus* (Compositae), and *Crotalaria retusa* (Leguminosae) plants. These species occasionally produce extremely high levels of PAs, up to 9 to 17% of leaf dry weight (23). In previously analyzed samples of *S. riddellii* and *S. longilobus* plants from New Mexico the PA contents were about 1.5 and 2.8%, respectively (10). Since there is little information about polamines, including Put, ADC, and ODC activities, or the site(s) of alkaloid biosynthesis in PA-bearing plants, and since such information is available only for *Heliotropium* (5, 7–9), a more detailed examination of PA-bearing plants seemed important. Flowering *S. riddellii* and *C. retusa* were chosen for this work.

**MATERIALS AND METHODS**

Plant Material. Seeds of *S. riddellii* were collected near Tucumcari, NM; young plants of *S. longilobus* were collected at the same location in late June 1986. Seeds of *C. retusa* P1-274951 were obtained from the Plant Materials Center U.S.D.A., Brooks ville, FL. The total alkaloid contents of the *S. riddellii* and *C. retusa* seeds were 1.4 and 2.1%, respectively, when calculated as monocrotaline. At sowing, the seeds of *C. retusa* were inoculated with nitrogen-fixing bacteria, 'EL' inoculant from Nitratin, Clearwater, FL. At flowering, dense oblong nodules with leghemoglobin in the center were found on the roots. In addition, three *Avena sativa* cultivars were used: Garry, a cross of Victory × (Victoria × Hajira Banner), Ogle, and Victory. The seeds of the first two cultivars were supplied by the Carolina Biological Company and the Victory seeds by Svalof AB, International, Svalöf, Sweden. The alkaloid-bearing plants were grown in the greenhouse as described previously (9); *C. retusa* plants did not receive N fertilizers.

At the stage of main shoot flowering six plants each of *S. riddellii* and *C. retusa* were sampled in two replicates. The organs were separated, weighed, cut, and stored frozen for analyses of basic amino acid, polamine, and alkaloid contents as well as for ODC and ADC activities. On the same day side shoots of *S. riddellii*, *S. longilobus*, and *C. retusa*—10, 14, and 8 shoots per treatment, respectively—were exposed to H2O, 2 mM DFMO, 2 mM DFMA, or both inhibitors together as described previously (5). After 24 h, 2 to 4 shoots per treatment were sampled and

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1 Abbreviations: PA, pyrrolizidine alkaloid; Put, putrescine; Spd, spermidine; Spm, spermine; Agm, agmatine; Orn, ornithine; Arg, arginine; ODC, ornithine decarboxylase; ADC, arginine decarboxylase; DFMO, D,L-a-difluoromethylornithine; DFMA, D,L-a-difluoromethylarginine; Rt, retention time; TLE, thin layer electrophoresis.
ORNITHINE DECARBOXYLASE AND PYRROLIZIDINE ALKALOIDS

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RESULTS

At flowering, S. riddelli and C. retusa plants resembled each

other in average PA content, 0.58 and 0.64% (as monocrotaline,

to

Dry weight, respectively (Table I). In both species the

relative PA levels in the stems were lower than those in the

leaves; the younger the stem or the leaves the higher was the PA

content. The two species differed, however, in PA accumulation in

the inflorescence (flower heads in Senecio) and roots; in S.

riddelli these organs exhibited the highest levels, whereas in C.

retusa they contained significantly less PAs than the youngest

leaves. After hydrolysis the alkaloids from both species yielded

only retronecine as the aminoalcohol moiety of the PAs; retronecine

was also the only necine found after hydrolysis of PAs from S.

longilobus shoots.

The levels of free Orn (Table II) were lower in S. riddelli than

in C. retusa, ranging from 1 to 3% that of PAs, and did not show

any great differences depending on the organ or its age. Free Arg

levels were also neither organ- nor age-dependent, except for

inflorescences which showed very high free Arg accumulation in

both species; free Lys exhibited similar behavior. Put, Spd, and

Spm were detected in all organs of both species. At the detecta-

bility threshold of 1.2 mmol/g fresh weight no measurable Agm

was found in any unhydrolyzed or hydrolyzed sample, except for

Crotalaria roots in which it amounted to 0.07 µmol/g dry weight.

Agm may have also occurred at a similar level in the

inflorescence of this species. However, due to the presence of

other compounds with Rt very close to that of Agm, its occur-

rence in the inflorescence is not certain. Diaminopropane was

not detected in either species; lysine-derived cadaverine was

detected only in C. retusa and ranged from traces to 0.04 µmol/g

dry weight.

In S. riddelli no conjugated polyamines were found in any

organ. The total of free Put and Put-derived Spd and Spm ranged

from 0.55 to 1.75 µmol/g dry weight as compared with 14 to 75

µmol/g dry weight of Put incorporated into the necines, remem-

bering that two molecules of Put give rise to one pyrrolizidine

molecule. Free Put accounted for 20 to 40% of total polyamines,

except for the roots where it amounted to about 70% of the total.

Inflorescences and leaves exhibited the highest polyamine levels.

No significant age-dependent differences in the polyamine levels

were found in stems or leaves.

In C. retusa, conjugated polyamines occurred only in the

inflorescence and roots, i.e. in the organs in which free polyamine

the remaining ones, with their stems in H2O, 1 mM DFMA, 1

mM DFMA, or both inhibitors together, were exposed in light to

2.5 mCl of 14C-labeled CO2 in a Plexiglas chamber as described in

Birecka and Catalafmo (8). After a 68 h exposure the shoots

were sampled, cut, and frozen in two replicates. In addition, each

of several shoots of S. riddelli and C. retusa was given 1 ml of

2 mM DFMO or DFMA labeled with about 1 µCi of D.L-α-[3,4-

3H]DFMO or D.L-α-[3,4-3H]DFMA, respectively. After the

solutions were absorbed (10-12 h) additional portions of 0.5 to 1.0

ml H2O were supplied to the test tubes; 24 h after the beginning

of exposure the immersed part of the stem was cut 1.5 cm above

the immersion level and sampled separately from the upper part

of the stem and the leaves.

Oat seedlings were grown in a mixture of vermiculite and peat

humus. The first leaves were sampled on the 5th d after sprouting

and assayed for ODC and ADC activities.

Analysis. Extraction, quantitation, and purification of alka-

loids, hydrolysis of labeled necine esters, recovery of labeled free

necines and their separation by TLC as well as recrystallization

were carried out as described in Birecka and Catalafmo (8). For

basic amino acid and polyamine analyses plant tissues were

homogenized with 6% HClO4. A portion of the homogenate was

 centrifugated at 12,000g and the supernatant was tested for free

amino acids and polyamines. Another portion of the homogenate

was hydrolyzed with 6 N HCl (final concentration) in sealed

ampules at 100°C for about 20 h. After centrifugation the hydro-

dryzed was dried in vacuo and the residue dissolved in 5% HClO4.

The amino acids were quantified by HPLC as described in

Birecka et al. (6) using an ion exchange Whatman SCX column.

Diaminopropane, Put, and cadaverine can also be separated on

this column. The Rt values for Orn, Lys, Arg, diaminopropane,

Put, and cadaverine were 15.6, 18.0, 22.2, 32.6, 35.4, and 40.8

min, respectively. Diaminopropane, Put, Spd, Spm, and cadaverine

were analyzed by dansylation. The derivatives were prepared as

follows: 0.2 ml of the extract was mixed with 0.15 ml

of 1.5 M Na2CO3/0.05 M Na2B4O7 (pH 10.7) buffer, 0.01 ml of

0.1 M dianinohexane (as an internal standard), and 0.9 ml of

1% dansyl chloride in acetone and the mixture was allowed to

stand at room temperature in the dark overnight. Excess proline

(0.075 ml of 15% solution) was added to the mixture and the

acetone was removed under a stream of N2. After addition of 0.6

ml H2O, 0.1 ml of 2 N NaOH, and 1 ml toluene the sample was

vigorously mixed for 30 s and then centrifuged. An aliquot (0.7

ml) of the toluene layer was transferred to a clean tube and

evaporated to dryness under N2. After addition of 0.7 ml of

acetoni6'he dansyl-polyamines were analyzed by HPLC on a

Beckman ODS-I column (4 × 250 mm, 5 µm) using a modification

of conditions described by Brown et al. (12). The deriva-

tives were detected with Kratos FS 950 fluorometer equipped

with 365 nm excitation and 428 nm emission filters. The mobile

phases consisted of buffer A, 0.02 M heptane sulfonic acid, and

buffer B, acetonitrile. The column was equilibrated with a mix-

ture containing 50% A and 50% B. After injection of a sample

buffer B was increased to 80% in 22 min and then to 100% in

10 min where it was maintained for additional 20 min; the

original conditions were then restored over 0.5 min. Another

sample was injected after equilibration at starting conditions for

10 min. The Rt values for diaminopropane, Put, cadaverine,

Spd, and Spm were 12.0, 12.6, 14.0, 22.8, and 29.4 min, respec-


tively.

Agm, which does not dansylate quantitatively, was assayed

after postcolumn derivatization with o-phthalaldehyde using an

Ultraspere ODS column as previously (6) and a buffer system

described by Seiler et al. (27); the Rt of Agm was 29.4 min.

Soluble protein was assayed using Coomassie blue and BSA as

the standard protein.
Table I. Weight, Soluble Protein, and Alkaloid Content of S. riddellii and C. retusa Plants

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Organ*</th>
<th>Fresh Weight</th>
<th>Dry Matter</th>
<th>Soluble Protein</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/plant</td>
<td>% fresh wt</td>
<td>mg/g fresh wt</td>
<td>µmol/g fresh wt</td>
<td>µmol/g dry wt</td>
</tr>
<tr>
<td>S. riddelli b</td>
<td>Main shoot</td>
<td>Stem</td>
<td>6.15</td>
<td>28.0</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>7.22</td>
<td>12.7</td>
<td>2.0</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>Inflorescence</td>
<td>2.48</td>
<td>22.0</td>
<td>3.6</td>
<td>8.26</td>
</tr>
<tr>
<td></td>
<td>Side shoots</td>
<td>Stems</td>
<td>2.45</td>
<td>24.1</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>5.22</td>
<td>14.0</td>
<td>2.4</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>Small roots</td>
<td>6.79</td>
<td>14.4</td>
<td>1.6</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. retusa</td>
<td>Main shoot</td>
<td>Stems</td>
<td>7.45</td>
<td>21.9</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>1.62</td>
<td>16.1</td>
<td>3.9</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Old leaves</td>
<td>1.62</td>
<td>16.1</td>
<td>3.9</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Mature leaves</td>
<td>5.58</td>
<td>17.4</td>
<td>5.6</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>Young leaves</td>
<td>0.66</td>
<td>18.9</td>
<td>6.9</td>
<td>6.95</td>
</tr>
<tr>
<td></td>
<td>Inflorescence</td>
<td>2.87</td>
<td>19.2</td>
<td>9.3</td>
<td>4.65</td>
</tr>
<tr>
<td></td>
<td>Side shoots</td>
<td>Stems</td>
<td>5.66</td>
<td>15.0</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>11.01</td>
<td>18.2</td>
<td>6.4</td>
<td>4.85</td>
</tr>
<tr>
<td></td>
<td>Small roots</td>
<td>6.41</td>
<td>9.0</td>
<td>3.1</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>Tap root</td>
<td>0.84</td>
<td>21.8</td>
<td></td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>42.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In S. riddellii, the tap root—0.69 g/plant—was not analyzed. In C. retusa, the old leaves started turning yellow; the sizes of the young leaves ranged from ½ to ½ that of the fully expanded leaves; the inflorescences included a few very small pods. In young plants collected in late June near Tucumcari, NM, the alkaloid contents of stems, leaves, and roots were 5.4, 16.2, and 13.1 µmol/g dry wt, respectively.

Table II. Basic Amino Acid and Polyamine Contents

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Organ</th>
<th>Free Amino Acids*</th>
<th>Free Polyamines*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Orn</td>
<td>Arg</td>
</tr>
<tr>
<td>S. riddelli b</td>
<td>Main shoot</td>
<td>Stem</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.39</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Inflorescence</td>
<td>0.23</td>
<td>5.61</td>
</tr>
<tr>
<td></td>
<td>Side shoots</td>
<td>Stems</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.31</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Small roots</td>
<td>0.15</td>
<td>1.58</td>
</tr>
<tr>
<td>C. retusa</td>
<td>Main shoot</td>
<td>Stem</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Old leaves</td>
<td>0.37</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>Mature leaves</td>
<td>0.35</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Young leaves</td>
<td>0.31</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Inflorescence</td>
<td>0.42</td>
<td>2.81</td>
</tr>
<tr>
<td></td>
<td>Side shoots</td>
<td>Stems</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.60</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>Small roots</td>
<td>0.72</td>
<td>1.44</td>
</tr>
</tbody>
</table>

*In the hydrolyzed homogenates from both species, Orn levels were similar to those found in the unhydrolyzed extracts; levels of Arg and Lys were over 60 and 100 µmol/g dry wt, respectively. No significant differences between hydrolyzed and unhydrolyzed samples were found either in the levels of Put or in levels of Spd and Spm except for inflorescences and roots of C. retusa plants. The Put, Spd, and Spm levels in hydrolyzed samples from inflorescences were 3.92; 5.42; and 0.94 µmol/g dry wt, respectively, and those from roots were 7.18; 6.04; and 3.02 µmol/g dry wt, respectively.
levels were at their highest. In these two organs total Put accounted for about 40% of total polyamines, whereas in other organs it constituted about 7 to 8% of the total. Again, stems exhibited a lower polyamine level than leaves; age-dependent differences were rather small.

At the detectability threshold of 0.1 to 0.2 nmol/h x g fresh weight at 0.5 mM Arg in the reaction mixture, TLE-based ADC activity was not found in any plant extract. No 14CO2 release was observed in ADC assays of dialyzed extracts or of the (NH4)2SO4 enzyme fractions from stems or inflorescence of C. retusa. In the remaining samples the 14CO2 release ranged between 0.5 and 5.1 nmol/h x g fresh weight but was not affected by 1 or 2 mM DFMA. ODC activity was assayed in dialyzed as well as (NH4)2SO4 fractions in phosphate buffer. The 14CO2 release during incubation with the (NH4)2SO4 enzyme fractions (Table III) was somewhat lower than during incubation with dialyzed crude extracts. However, significant differences could still be found between TLE-based activities and those based on 14CO2 released, especially in S. riddellii. These differences cannot be explained by the presence of phenolics (21) or by the production of H2O2 by amine oxidases (1, 22). Due to very low ODC activities in some organs and the high cost of [U-14C]Orn the assays were carried out at 0.5 mM Orn in the reaction mixtures. When tested at 1 mM Orn, the activity of ODC from side shoot leaves was about 30% higher than that determined at 0.5 mM Orn.

In S. riddellii the enzyme activities per dry weight were significantly higher than those in C. retusa. The lowest in vitro activity was found in stems and the highest in leaves, especially in those of the side shoots. The activities in inflorescence and roots were about 30 and 12%, respectively, of that found in leaves. In C. retusa ODC activity was barely detectable in stems, very low in the inflorescence, roots, and old leaves, and relatively high in young leaves. The TLE-based in vitro ODC activities could be completely suppressed by 1 mM DFMO.

The concentration and radioactivity of 14CO2 in the chamber and the light conditions in this study were similar to those used in previous experiments and the exposure lasted 68 instead of 44 h. In spite of this, the radioactivity here per g of shoot fresh weight, particularly in the two Senecio species (Table IV), was much lower than it had been in the borage or in S. vulgaris. The inhibitors had no significant effect either on the rate of 14CO2 assimilation or on the transformation of the assimilates into ethanol-insoluble compounds. Enzyme activity assays were performed in the detached side shoots immediately prior to 14CO2 exposure, i.e. 24 h after they were treated with the inhibitors. As shown in Table V, S. longilobus shoots, in which no measurable ADC activity could be detected, revealed a somewhat higher ODC activity than did S. riddellii. DFMO almost completely inhibited the enzyme in all three species and in neither species did DFMA have any significant effect on ODC activity as reported for tobacco (28).

The relative incorporation of 14C into retronecine in the control shoots of the Senecio species studied here was much higher than in S. vulgaris, but significantly lower than in Heliotropium shoots with a higher PA content. As shown, DFMA had no effect on 14C incorporation into the necine, whereas DFMO lowered it very significantly. When twice recrystallized, retronecine from control shoots of the tested species did not change significantly in specific radioactivity. Neither were any marked changes observed in the twice recrystallized retronecine from DFMO-treated S. riddellii shoots in which the radioactivity amounted to about 35% that of the control; a similar level of necine radioactivity was found in shoots treated with both inhibitors. In the two other species, the relative 14C incorporation into retronecine from shoots treated with DFMO alone or together with DFMA amounted to about 20% of that in the controls. In the borage, the necine radioactivities in plants treated with DFMA alone or together with DFMO accounted for only 3 to 4% of that found in the controls.

The distribution of 3H-labeled DFMO and DFMA in the treated shoots (Table VI) indicates that the inhibitors were not strongly retained by the stem xylem along which they apparently moved. In S. riddellii and C. retusa the leaves contained about 50 and 70%, respectively, of the inhibitors applied. The higher transpiration of Crotalaria leaves might be responsible for the

<p>| Table III. ODC Activities in the Presence of 0.5 mM L-[U-14C]Orn and 0.1 mM Aminoguanidine |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Organ</th>
<th>14CO2 Released*</th>
<th>Activity Based on [U-14C] Put after TLE nmol/h·g fresh wt</th>
<th>nmol/h·g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. riddellii</td>
<td>Main shoot</td>
<td>Stem</td>
<td>2.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>7.5</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflorescence</td>
<td>7.3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Side shoots</td>
<td>Stem</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>11.1</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>Small roots</td>
<td>2.4</td>
<td>1.0</td>
<td>6.9</td>
</tr>
<tr>
<td>C. retusa</td>
<td>Main shoot</td>
<td>Stem</td>
<td>0.3</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Old leaves</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature leaves</td>
<td>4.1</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Young leaves</td>
<td>6.0</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflorescence</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Side shoots</td>
<td>Stems</td>
<td>0.3</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaves</td>
<td>5.2</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Small roots</td>
<td>1.0</td>
<td>0.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* The rates of 14C release from [1-14C]Orn were similar to those from [U-14C]Orn.
greater import of the inhibitors in this species. TLE results did not indicate any detectable changes of the inhibitors in the plants.

The negative results of the ADC assays prompted us to reexamine oat cv Garry that previously exhibited very high ADC activities, 334 and 1096 nmol/h x mg protein, in the first leaf of seedlings grown in the greenhouse and on a windowsill, respectively; the Put-based ODC activity was about 0.2 to 0.3 nmol/h x mg protein in both cases (6, 7). It was worrisome that the activities of the two enzymes reported for comparable leaves of oat cv Victory seedlings, grown under controlled conditions, were much different from ours and at the same time showed significant variability. The reported ADC and ODC activities varied from 2.2 to about 32 and from 0.8 to 18 nmol/h x mg protein, respectively, with ODC/ADC ratios ranging from 0.1 to 6.0 (16, 19, 31, 33, 34). Thus, one might suppose that there is significant intraspecific variation in activity levels and/or substantial dependence on external uncontrolled factors; e.g. Put-based ODC activity is known to respond to infection (24) and ADC activity responds sharply to stress (17, 29).

As shown in Table VII, leaves of cv Garry seedlings grown from a new batch of seeds exhibited an ADC activity similar to that previously found under the same conditions while their ODC activity was somewhat higher. Compared to Garry, the other two cultivars showed significantly lower ADC and rather similar ODC activities; however, in both cases the ADC activity was dominant. In view of the consistent dominance of ADC in these oat varieties, one would like to assume that results for the wild plants studied here are characteristic for their species.

### Table IV. Fresh Weight and Radioactivity of Shoots

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Treatment</th>
<th>Fresh Wt*</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EtOH solube</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fraction</td>
</tr>
<tr>
<td>S. riddelli</td>
<td>H2O</td>
<td>1.57</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>DFMO</td>
<td>1.64</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>DFMA</td>
<td>1.74</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>DFMO + DFMA</td>
<td>1.83</td>
<td>11.9</td>
</tr>
<tr>
<td>S. longilobus</td>
<td>H2O</td>
<td>1.32</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>DFMO</td>
<td>1.24</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>DFMA</td>
<td>1.34</td>
<td>11.7</td>
</tr>
<tr>
<td>C. retusa</td>
<td>H2O</td>
<td>2.93</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>DFMO</td>
<td>2.79</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>DFMA</td>
<td>2.72</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>DFMO + DFMA</td>
<td>2.94</td>
<td>12.2</td>
</tr>
</tbody>
</table>

* The dry matter of S. riddelli, S. longilobus, and C. retusa shoots was 16.2, 14.0, and 17.6% of fresh weight, respectively.

### Table V. ODC Activity, Alkaloid Content, and Ncine Radioactivity in Shoots Exposed to 14C-Labeled CO2

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Treatment</th>
<th>ODC Activity</th>
<th>Labeled Alkaloid Content before Hydrolysis</th>
<th>Necine Recovered after Hydrolysis</th>
<th>Radioactivity of Necine fraction after hydrolysis</th>
<th>Retronecine after TLE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmol/h-g fresh wt</td>
<td>µmol/g fresh wt</td>
<td>10^3 cpm/g fresh wt</td>
<td>% total radioactivity</td>
<td></td>
</tr>
<tr>
<td>S. riddelli</td>
<td>H2O</td>
<td>6.0</td>
<td>3.6</td>
<td>3.5</td>
<td>47.6</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>DFMO</td>
<td>&lt;0.2</td>
<td>3.7</td>
<td>3.4</td>
<td>20.1</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>DFMA</td>
<td>5.9</td>
<td>3.3</td>
<td>3.0</td>
<td>43.2</td>
<td>20.1</td>
</tr>
<tr>
<td></td>
<td>DFMO + DFMA</td>
<td>&lt;0.2</td>
<td>3.5</td>
<td>3.4</td>
<td>16.9</td>
<td>7.2</td>
</tr>
<tr>
<td>S. longilobus</td>
<td>H2O</td>
<td>7.1</td>
<td>4.6</td>
<td>5.0</td>
<td>29.6</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>DFMO</td>
<td>&lt;0.2</td>
<td>4.5</td>
<td>4.0</td>
<td>25.9</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>DFMA</td>
<td>7.2</td>
<td>5.5</td>
<td>5.0</td>
<td>36.0</td>
<td>19.4</td>
</tr>
<tr>
<td>C. retusa</td>
<td>H2O</td>
<td>3.8</td>
<td>4.5</td>
<td>4.3</td>
<td>74.6</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td>DFMO</td>
<td>&lt;0.2</td>
<td>4.5</td>
<td>4.0</td>
<td>25.9</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>DFMA</td>
<td>3.6</td>
<td>4.3</td>
<td>4.0</td>
<td>81.6</td>
<td>28.7</td>
</tr>
<tr>
<td></td>
<td>DFMO + DFMA</td>
<td>&lt;0.2</td>
<td>4.8</td>
<td>4.2</td>
<td>29.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* The plants were not assayed either for basic amino acids or for polyamines. No measurable TLE-based ADC activity was found.
Table VI. Distribution of D,L-α-[3,4-³H]DFMO and D,L-α-[3,4-³H]DFMA in Shoots Exposed to the Inhibitors at 2 mM for 24 h

<table>
<thead>
<tr>
<th>Plant</th>
<th>Organ</th>
<th>DFMO</th>
<th>DFMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. riddellii</td>
<td>Leaves</td>
<td>53.3</td>
<td>50.5</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>22.8</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>23.5</td>
<td>27.4</td>
</tr>
<tr>
<td></td>
<td>Immersed</td>
<td>11.1</td>
<td>12.5</td>
</tr>
<tr>
<td>C. retusa</td>
<td>Leaves</td>
<td>73.8</td>
<td>73.0</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>11.1</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Upper</td>
<td>15.1</td>
<td>14.3</td>
</tr>
</tbody>
</table>

*The fresh weights of the leaves, the stem upper portion, and the immersed stem were about 0.75, 0.35, and 0.18 g per shoot, respectively, in S. riddellii, and 0.96, 0.32, and 0.17 g, respectively, in C. retusa.

*The total radioactivity of DFMO or DFMA applied was about 3.6 × 10⁶ cpm per shoot; the radioactivity found in the solutions left in the test tubes at the end of the experiment ranged from 0.4% of the total.

Table VII. ADC and ODC Activities in Oat Leaves

<table>
<thead>
<tr>
<th>Oat Cultivar*</th>
<th>ADC Activity Based on ¹⁴CO₂</th>
<th>ODC Activity Based on ¹⁴CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[¹⁴CO₂]Put</td>
<td>[¹⁴CO₂]</td>
</tr>
<tr>
<td>Garry</td>
<td>373</td>
<td>0.7</td>
</tr>
<tr>
<td>Victory</td>
<td>48</td>
<td>0.9</td>
</tr>
<tr>
<td>Ogle</td>
<td>88</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*The dry matter in leaves of the tested cultivars amounted to about 12% of the fresh weight; the soluble protein contents ranged from 4.3 to 4.5 mg/g fresh weight.

DFMA at 1 mM inhibited over 98% of the ADC activity in all cultivars.

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Acknowledgments—We are grateful to Mr. Ted E. Peabody and Mrs. S. A. Crane, T. J. Tanumari, NM, for their extraordinary help in collecting live plants and seeds of S. longifolius and S. riddellii. We also thank Mr. S. Stavekels and A. Crowely for their very effective assistance during the experiments.

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