more fully oxygenated) medium suppresses formation of slaframine relative to pyrindine. We have repeated this experiment and find that, whereas the combined yield of slaframine and deacetylslaframine is typically 2.2 times that of the pyridine in still cultures, the yields are approximately equal with agitated cultures.

It is interesting from the standpoint of metabolic relationships of host and parasite that *R. leguminicola* should produce characteristic secondary metabolites from two compounds which are characteristic constituents of red clover: pipecolic acid (Greenstein & Winitz, 1961) and malonic acid (Soldatenko & Mazurova, 1957).

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Biosynthesis of Slaframine, (1*S*,6*S*,8a*S*)-1-Acetoxy-6-aminooctahydroindolizine, a Parasympathomimetic Alkaloid of Fungal Origin. 4. Metabolic Fate of Ethyl Pipecolylacetate, 1,3-Dioxooctahydroindolizine, and 1-Hydroxyoctahydroindolizine in *Rhizoctonia leguminicola*†

E. C. Clevenstine, P. Walter, T. M. Harris,* and H. P. Broquist*

ABSTRACT: Known or suspected intermediates in the biosynthesis of slaframine and 3,4,5-trihydroxyoctahydro-1-pyrindine, piperidine alkaloids of the phytopathogenic fungus *Rhizoctonia leguminicola*, were prepared and tested for biological conversions. Ethyl pipecolylacetate, an analogue of the postulated condensation product of pipecolic and malonic acids (two previously identified alkaloid precursors), was insufficiently stable for feeding experiments. The lactam of pipecolylacetate, 1,3-dioxooctahydroindolizine, was degraded by the fungus without direct incorporation into alkaloids. The known slaframine precursor 1-hydroxyoctahydroindolizine was prepared by a novel route which permitted high levels of deuterium enrichment at C-1 and C-3. Mass spectrometric examination of the slaframine biosynthesized from *cis*- and *trans*-[1,3,3-^{2}H]-1-hydroxyoctahydroindolizine strengthened arguments that 1-oxooctahydroindolizine is an intermediate in slaframine biogenesis.

Lysine is converted by the phytopathogenic fungus *Rhizoctonia leguminicola* to two unusual piperidine alkaloids: 1-acetoxy-6-aminooctahydroindolizine (slaframine, **1**) and 3,4,5-trihydroxyoctahydro-1-pyrindine (**2**) (Aust & Broquist, 1965; Guengerich et al., 1973a). As indicated by the heavy lines in the structural drawings in Scheme I, six of the eight carbons in both compounds are derived from the six carbons of lysine via pipecolic acid (Guengerich et al., 1973a,b). In isotope incorporation experiments described in the previous communication in this series (Clevenstine et al., 1979), it has been shown that the remaining two carbons in the five-membered rings of both **1** and **2** are derived from acetate via malonate. These observations are incorporated in the proposed biosynthetic pathway to slaframine shown in Scheme I.

This paper describes two attempts to define further the early stages of slaframine biosynthesis. The first involves feeding ethyl pipecolylacetate (**7**), which is closely related to **3**, the hypothetical common precursor of alkaloids **1** and **2**. The second involves the lactam 1,3-dioxooctahydroindolizine (**4**), which might itself be an intermediate in the pathway or, alternatively, might be able to enter the pathway by conversion to **3**.

During the course of this work a facile synthesis of the 1-hydroxy derivatives (**5a** and **5b**) of octahydroindolizine

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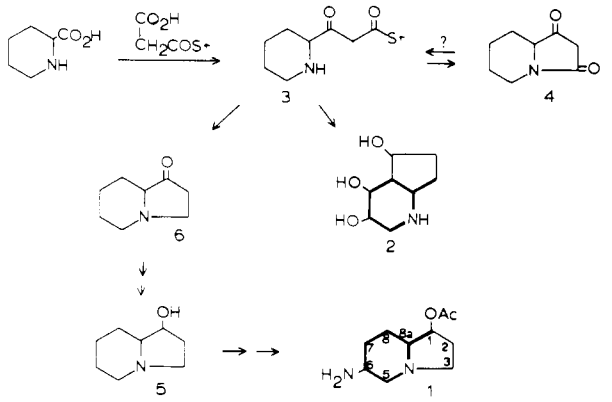
Scheme I: Hypothetical Pathway for Biosynthesis of Slaframine (1) and 3,4,5-Trihydroxyoctahydro-1-pyrindine (2) in *R. leguminicola*



(OHI)[1] was found which involves reduction of lactam **4**. Previous work (Guengerich et al., 1973b) had shown that 1-oxo-OHI (**6**) and 1-hydroxy-OHI (**5**) could serve as precursors of slaframine. Reduction of 1-oxo-OHI by partially purified extracts of *R. leguminicola* was demonstrated to give *cis*-1-hydroxy-OHI (**5a**), which is consistent with the relative configuration of the 1-acetoxy group of slaframine (Gardiner et al., 1968). However, when [6,7-^{3}H]-*cis*- and [6,7-^{3}H]-*trans*-1-hydroxy-OHI were incubated with resting cells of *R. leguminicola*, both isomers were converted to slaframine. We have now reinvestigated the metabolism of the cis and trans alcohols (**5a** and **5b**) using deuterium substituents and have found that the trans compound initially undergoes oxidation to the 1-oxo analogue (**6**) before entering the biosynthetic pathway.

Materials and Methods

Syntheses. Melting points were taken with Fisher-Johns or Fisher Meltemp devices. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. NMR spectra were obtained with a JEOL MH-100 spectrometer with tetramethylsilane as an internal standard using external lock. Mass spectra (electron impact, 70 eV) were obtained by direct introduction of samples into an LKB-9000A mass spectrometer. Thin-layer chromatography was carried out with silica gel or cellulose plates, compounds being visualized with ninhydrin (0.2% in absolute EtOH) and/or iodine vapor.

Ethyl DL-*Pipecolylacetate* (**7**). Condensation of ethylmalonyl chloride with benzyl alcohol in the presence of *N*,*N*-dimethylbenzylamine gave ethyl benzyl malonate in 67% yield after distillation: bp 135–145 °C (0.75 mm); $n^{22.8}{}_D$ 1.44880; IR (neat) 3050 (w), 2970 (m), 1760 (vs), 1740 (vs) cm^{-1}; NMR ($CDCl_3$) δ 7.36 (s, $C_6H_5CH_2$), 5.16 (s, $C_6H_5CH_2$), 4.13 (q, J = 8 Hz, CH_2CH_3), 3.42 (s, CH_2), 1.14 (t, J = 8 Hz, CH_2CH_3); lit. bp 108–111 °C (0.33 mm) (Dahn & Hauth, 1959). In order to form the magnesium salt of ethyl benzyl malonate, the ester (2.75 g, 12.3 mmol) was added to a refluxing mixture of finely clipped Mg ribbon (296 mg), CCl_4 (0.2 mL), EtOH (1.45 mL), and tetrahydrofuran (10 mL) under N_2. The mixture was evaporated to dryness in vacuo after dissolution of the Mg was complete.

DL-Cbz-pipecolic acid (mp 171–172 °C after recrystallization from water) was prepared by acylation of DL-pipecolic acid with carbobenzoxy chloride in the presence of Mg. Ethyl chloroformate (1.2 mL, 12 mmol) in tetrahydrofuran (5 mL) was added to a mixture of Cbz-pipecolic acid (3.09 g, 11.8 mmol) and triethylamine (1.23 g, 12.3 mmol) in tetrahydrofuran (30 mL) at −20 °C with vigorous agitation. The mixture was warmed to 0 °C and, after 10 min, the Mg salt of ethyl benzyl malonate from above was added in tetrahydrofuran (10 mL). After standing overnight, the product mixture was partitioned between diethyl ether and cold, dilute H_2SO_4. The organic phase was washed with water, dried over Na_2SO_4, and evaporated. The residue was washed with cyclohexane to remove unreacted ethyl benzyl malonate, leaving 610 mg (11%) of the pipecolate–malonate adduct as a clear yellow oil, which solidified on standing (mp 60–61 °C).

Hydrogenation (3 atm, ambient temperature) of the adduct in ethanol (1 mL) containing 10% Pd/C (40 mg) and acetyl chloride (157 mg) yielded 271 mg (88%) of ethyl pipecolylacetate hydrochloride (**7**): mp 233 °C (sublimation above 200 °C); IR (KBr) 3380 (m), 1750 (vs), 1640 (m), 1580 (m) cm^{-1}; NMR (free base in $CDCl_3$) δ 5.35 (s, −CH= of enol form), 3.40 (m, CH_2 of keto form), 4.22 (q, J = 7 Hz, CH_2CH_3), 2.06 and 1.53 (multiplets, piperidyl), 1.32 (t, J = 7 Hz, CH_2CH_3). Treatment of **7** with 1% methanolic ferric chloride gave a red color with absorption maximum at 490 nm, characteristic of open-chain β-keto esters (Henecka, 1950). The compound can be monitored by chromatography on cellulose TLC plates with development in 95% ethanol or 75% 1-propanol mixtures with water. Compound **7** had R_f values of 0.52 in 95% ethanol and 0.53 in 75% 1-propanol; by comparison, pipecolic acid had R_f values of 0.33 and 0.39, respectively, in the two solvents. On standing in water, **7** underwent gradual hydrolysis to give pipecolic acid; hydrolysis was essentially complete after 24 h at room temperature.

Ethyl pipecolylacetate was also synthesized with ^{14}C label at the 2 position of the side chain. For the synthesis, diethyl [2-^{14}C]malonate (New England Nuclear) diluted with unlabeled ester was hydrolyzed to methylmalonic acid and converted via the acid chloride to ethyl benzyl [2-^{14}C]malonate. Treatment as above with Cbz-pipecolic acid gave **7** hydrochloride (specific activity 5.0 μCi/mmol).

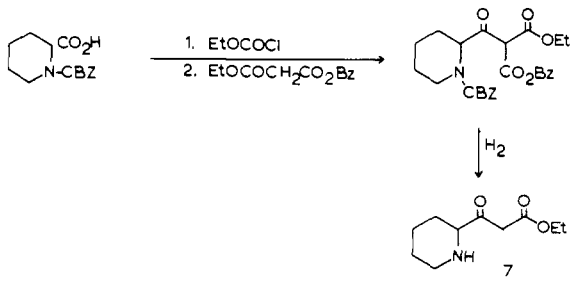
DL-*1,3-Dioxooctahydroindolizine* (**4**). A solution of ethyl DL-*N*-acetylpipecolate (3 g, 15 mmol) (Doyle et al., 1964) in 25 mL of tetrahydrofuran was added dropwise to a stirred suspension of pentane-washed potassium hydride (1.53 g, 38 mmol) in 50 mL of tetrahydrofuran under nitrogen. After 3 h of stirring at room temperature, the reaction mixture, which contained a flocculent, light yellow precipitate, was evaporated to dryness and the residue was quickly taken up in 100 mL of ice–water. The solution was acidified to pH 6 with cold, dilute HCl and extracted with 150 mL of methylene chloride in small portions. The organic phase was dried over magnesium sulfate and evaporated in vacuo to give 1.56 g (68%) of 1,3-dioxo-OHI (**4**) which gave a single peak on GLC (3% OV-17 on Chromosorb W, 2 mm × 2 m, retention time 1.4 min at 170 °C and 60 mL/min He flow): IR (neat) 1690 (lactam CO), 1770 (keto CO) cm^{-1}; NMR ($CDCl_3$) δ 3.0 (s, 2-CH_2), remaining signals were unresolved multiplets in the range 1.2–4.4; MS m/e (%) 153 (14, parent), 125 (96), 97 (79), 82 (100), 68 (15), 55 (100). Anal. ($C_8H_{11}NO_2$) C, H.

Efforts to distill **4** led to extensive formation of aldol-type dimers and higher polymers. Dimerization could also be observed during TLC on silica gel plates (ethyl acetate:pentane:acetic acid, 75:25:1; R_f of **4** = 0.5) unless the TLC plate was moistened with eluting solvent, while the material was spotted.

Two radioisotopically substituted samples of **4** were prepared by cyclization of labeled ethyl *N*-acetylpipecolate samples. Piperidine ring-tritiated **4** was prepared from (*R*)-[^{3}H]pi-

[1] Abbreviations used: OHI, octahydroindolizine; Cbz, carbobenzoxy.

Scheme II: Synthesis of Ethyl Pipecolylacetate (7)



pecolate. Isotopic carbon (^{14}C) was introduced into the pyrrolidine ring by adding unlabeled ethyl pipecolate to acetic anhydride which had previously been equilibrated with sodium [1-^{14}C]acetate. The procedure, although isotopically inefficient, provided a convenient route to [3-^{14}C]-1,3-dioxo-OHI.

DL-*1-Hydroxyoctahydroindolizine* (**5**). 1,3-Dioxo-OHI (**4**) (1 g, 6.5 mmol) in 10 mL of dry tetrahydrofuran was added dropwise under nitrogen to a suspension of $LiAlH_4$ (1 g, 24 mmol) in 200 mL of dry diethyl ether. The mixture was refluxed 2 h. Water (1 mL) was added dropwise to the cooled reaction mixture with vigorous agitation, followed by 1 mL of NaOH solution (3 M) and 3 mL of water. The crystalline precipitate was filtered off and washed with 20 mL of boiling diethyl ether. The combined ether phases were dried over magnesium sulfate, and the solvent was evaporated to yield 0.6 g of a slightly yellow oil. The two isomers of 1-hydroxy-OHI (**5**) were separated by gas–liquid chromatography as described by Aaron et al. (1966). On a 9 mm × 3 m column of 15% Carbowax 20 M on Chromosorb W at 200 °C with He flow of 120 mL/min, the cis alcohol (**5a**) eluted after 6.3 min and the trans alcohol (**5b**) after 9.2 min. The two products were collected in condenser tubes cooled to −78 °C. Repeated injections afforded 160 mg of *cis*- and 230 mg of *trans*-OHI (65% total yield). NMR spectra of the samples of **5a** and **5b** were identical with those reported by Gardiner (1970).

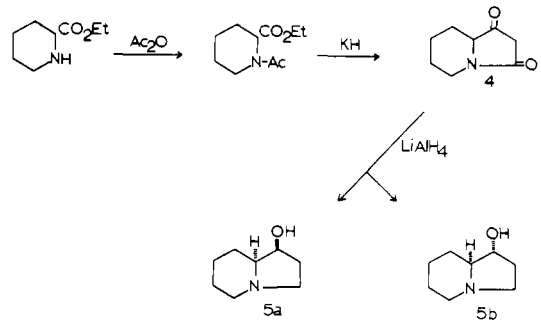
Reduction of 1,3-dioxo-OHI (**4**) using $LiAl^2H_4$ in the procedure described above yielded cis and trans isomers of 1,3,3-trideuterio-1-hydroxy-OHI with mass spectra showing molecular ions at *m/e* 144, corresponding to an increase of 3 mass units above the molecular weight of the undeuterated alcohols. The NMR spectra of the deuterated compounds showed the expected simplifications. In the case of the cis isomer, the multiplet at 4.05 ppm disappeared and the multiplet at 3.1 ppm became resolved into a broad doublet, supporting Gardiner's (1970) assignment of the spectrum of the undeuterated compound. These effects appeared at 3.9 and 3.0 ppm, respectively, in the spectrum of the trans isomer.

Biological Experiments: Incubation of R. leguminicola with Synthetic Compounds. Cultivation of the fungus, feeding of precursors, and isolation of slaframine were carried out as described by Guengerich & Broquist (1973), with the minor modifications mentioned in the accompanying paper (Clevenstine et al., 1979).

Results and Discussion

Syntheses. Ethyl DL-*Pipecolylacetate* (**7**). DL-Pipecolic acid was the starting point for the synthesis of keto ester **7** (Scheme II). Initial protection of the secondary amino group was required prior to activation of the carboxyl group to avoid a facile dimerization to form the corresponding diketopiperazine. The carbobenzoxy (Cbz) group was chosen for this purpose because of its stability to nucleophilic attack coupled with its ease of removal by hydrogenolysis. By using a procedure modeled upon reaction sequences described by Breslow et al. (1944) and by Price & Tarbell (1963), the carboxyl group of

Scheme III: Syntheses of DL-1,3-Dioxo-OHI (**4**) and the Cis (**5a**) and Trans (**5b**) Isomers of DL-1-Hydroxy-OHI



Cbz-pipecolic acid was activated by treatment with ethyl chloroformate. The resulting mixed anhydride was used to acylate the magnesium salt of ethyl benzyl malonate. Hydrogenolysis of the resulting acylmalonate under acidic conditions cleaved both benzyl esters; spontaneous decarboxylations gave the desired β-keto ester **7**.

It was anticipated that ester **7** might hydrolyze readily, but the degree of sensitivity was not apparent until after radioactive material had been prepared and incubated with *R. leguminicola*. It was subsequently shown by periodic thin-layer chromatography of samples taken from an aqueous solution of the hydrochloride of **7** that hydrolysis occurred rapidly and was complete in less than 24 h at room temperature. The principal hydrolysis reaction involves cleavage of the pipecolyl group rather than of the ester group; pipecolic acid was identified as the cleavage product by using thin-layer chromatography.

1,3-Dioxooctahydroindolizine (**4**). Compound **4** was prepared by cyclization of ethyl *N*-acetylpipecolate in the presence of potassium hydride (Scheme III). The pure compound was unstable, readily undergoing aldol-type intermolecular condensations between 1-oxo and 2-methylene groups. The dimer was characterized by its mass spectrum which showed a molecular ion at *m/e* 288. Compound **4** was stable in water, ethanol, methanol, methylene chloride, and chloroform. Aqueous solutions of **4** at pH 6 were stored at room temperature for 3 days. At intervals, aliquots were extracted with methylene chloride; gas chromatographic analysis of the extracts revealed no degradation of the compound.

A less direct route to **4** based on the work of Stork & Szajewski (1974) was investigated cursorily prior to the synthesis by the route outlined above. Acylation of ethyl pipecolate with ethylmalonyl chloride followed by cyclization, hydrolysis, and decarboxylation failed to give detectable **4**, although it is probable that the result can be ascribed to our failure to recognize the instability of the product.

1-Hydroxyoctahydroindolizine (**5**). Compound **4** was reduced with $LiAlH_4$ in tetrahydrofuran (Scheme III). The resulting alcohol was a mixture of the cis and trans isomers which could be separated readily by preparative GLC (Aaron et al., 1966). The reduction of **4** was repeated with $LiAl^2H_4$, yielding the 1,3,3-trideuterio derivative of **5**. The isomers were again separated by preparative GLC. This route to **5** has several distinct advantages over an earlier procedure of Clemo & Ramage (1932). The acetylation of ethyl pipecolate with acetic anhydride is much easier to carry out than the alkylation with 3-chloropropionate. Cyclization of ethyl *N*-acetylpipecolate gives 1,3-dioxo-OHI ready for reduction, whereas the other route requires additional steps. The new method is much better suited to introduction of isotopic labels into the pyrrolidine ring. In the present study deuterium substitution

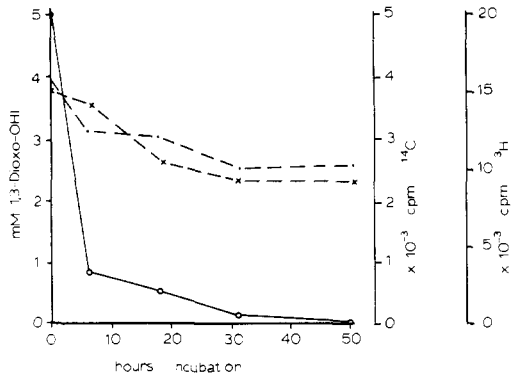


FIGURE 1: Concentration of 1,3-dioxo-OHI (**4**) and amount of radioactivity during incubation of *R. leguminicola* with [3-^{14}C,5,6,-7,8-^{3}H]-1,3-dioxo-OHI as a function of incubation time. (O—O) Concentration of **4**; (×– –×) cpm of ^{14}C/25 μL of medium; (·– –·) cpm of ^{3}H/25 μL of medium.

Table I: Relation of Synthetic Compounds to the Slaframine Pathway[a]

precursors	sp act. (μCi/mmol) pre-cursor	sla-framine	sp act. ratio, slaframine/ precursor
2.8 mM (*R*)-[^{3}H]pipecolic acid	9.9	0.81	8.2×10^{-2}
2.8 mM (*R*)-[^{3}H]pipecolic acid + 15.5 mM 1,3-dioxo-OHI	9.9	0.48	4.8×10^{-2}
5.2 mM [3-^{14}C]-1,3-dioxo-OHI	28	0.082	2.9×10^{-3}
1.0 mM [1-^{14}C]malonic acid	50	0.22	4.4×10^{-3}
0.5 mM [1-^{14}C]acetic acid	50	0.12	2.5×10^{-3}

[a] 1,3-Dioxo-OHI (**4**) was synthesized with and without radioactive labels, as described under Materials and Methods. Confluent *R. leguminicola* mycelial mats were resuspended in 50-mL portions of the solutions listed above under "precursors". Mycelium was harvested after 2 days of incubation; slaframine was isolated and its specific activity determined as described under Materials and Methods.

was required at two positions in **5**, the 1 position because this was the site under investigation and a passive location such as position 3 to provide an indication of the extent of incorporation of the labeled **5** even if label were lost from the 1 position.

Biological Experiments: Metabolism of **7** *and* **4**. As mentioned above, the rapid hydrolysis of **7** in the absence of fungus negated the value of feeding experiments.

It was hoped that the β-keto compound could be implicated in slaframine biogenesis through its lactam, 1,3-dioxooctahydroindolizine (**4**). Gas chromatographic analysis of CH_2Cl_2 extracts of aqueous solutions of **4** containing 5 mM pipecolic acid indicated that **4** was stable for at least 48 h at room temperature. However, when medium was assayed periodically during an incubation of **4** with *R. leguminicola*, the concentration of **4** fell almost 90% in 6 h (Figure 1).

Lines 1 and 2 of Table I show that unlabeled lactam significantly reduces incorporation of (*R*)-[^{3}H]pipecolic acid into slaframine. Unfortunately, ^{14}C at the 3 position of **4** was not incorporated efficiently, the level not exceeding that of acetate and malonate (lines 3–5). The question, whether the failure to incorporate label was due to an inability of the fungus to metabolize **4** once it had taken the compound up, was answered by incubating the fungus with **4** labeled with ^{3}H in the piperidine ring and with ^{14}C in the 3 position. Ion-exchange chromatography of cell extracts showed a large peak of ^{3}H activity with no associated ^{14}C peak eluting at the position of pipecolic acid in standard profiles. Carrier pipecolic acid was added to the corresponding fractions and recovered by crystallization. Radioactivity was retained by the pipecolic acid on repeated recrystallization, confirming that the double-labeled lactam was degraded metabolically to liberate pipecolic acid. The absence of ^{14}C activity showed that the pipecolate did not arise by resynthesis. The failure of the lactam to act as a direct precursor of slaframine is probably not surprising, in view of the difficulty of reducing the amide function to a tertiary amine.

The failure of ethyl pipecolylacetate to be converted to slaframine is not without precedent. Indirect but nonetheless compelling evidence has been obtained that poly-β-keto esters are intermediates in the biosynthesis of many aromatic secondary metabolites (Richards & Hendrickson, 1964), but incorporation experiments with keto acids have met with failure. For example, 3,5-dioxohexanoic acid was not utilized directly in formation of the fungal metabolite 6-methylsalicylic acid (Light et al., 1966). The diketo acid underwent initial degradation to acetate which then entered the pathway in a normal fashion. Enzymes which cleave β-keto acids (Connors & Stotz, 1946; Brock & Williamson, 1968) and enol lactones of diketo acids (Light et al., 1966; Meister, 1949) have been found previously.

Enol lactones of poly-β-keto acids have been isolated from living systems. In some cases these lactones have been implicated as shunt products of metabolic pathways normally leading to aromatic products. However, a search for **4** in fungal extracts was not fruitful.

The experiments described herein have failed to identify the first intermediate in the slaframine pathway which contains all the skeletal carbon atoms. In all probability, this intermediate is an enzyme-bound ester **3** but the inability of **4** and **7** to enter the pathway will thwart attempts to establish this intermediate until viable enzyme preparations have been obtained. Interception of enzyme-bond β-keto ester with nucleophiles should be possible in cell-free systems.

*Metabolism of cis- and trans-***5**. Two mature cultures of *R. leguminicola* were suspended on sterile water containing *trans*-[1,3,3-^{2}H]-1-hydroxy-OHI. Two other cultures were likewise incubated with solutions of the cis isomers. After 2 days of incubation, slaframine was isolated from the cultures and subjected to mass spectrometric analysis. Figure 2 shows selected regions of the mass spectra of the products of these incubations, compared with unlabeled slaframine. The presence of substantial isotopic satellite peaks in the mass spectrum of slaframine and deacetylslaframine obtained from both isomers confirmed the earlier observation (Guengerich et al., 1973b) that both isomers of **5** were effective precursors of slaframine. Incorporation of the cis compound (**5a**) was more efficient than the trans (**5b**). For the most part, **5a** was used directly, while **5b** had to be converted initially to ketone **6**. As shown in Figure 2, trideuterio **5a** is metabolized mainly without loss of deuterium, whereas at least three-quarters of the slaframine derived from trideuterio **5b** has lost one deuteron. The deuteron lost from **5b** comes from the 1 position. The mass spectrum of slaframine contains an intense fragment at *m/e* 70 ($C_4H_8N^+$) which was originally attributed by Gardiner et al. (1968) to the pyrrolidine ring (bridgehead nitrogen plus carbons 1, 2, 3, and 8a), but subsequent study of model compounds led Gardiner (1970) to conclude that the ion with *m/e* 70 is actually derived from the bridgehead nitrogen plus carbons 3, 5, 8, and 8a. The latter assignment received confirmation from the $C^2H_3CO_2H$ feeding experiment described in the accompanying paper (Clevenstine et al., 1979), which yielded slaframine containing two deuterons at C-2. The *m/e* 70–72 region of the mass spectrum of that material showed no significant enrichment. Both of the present enriched

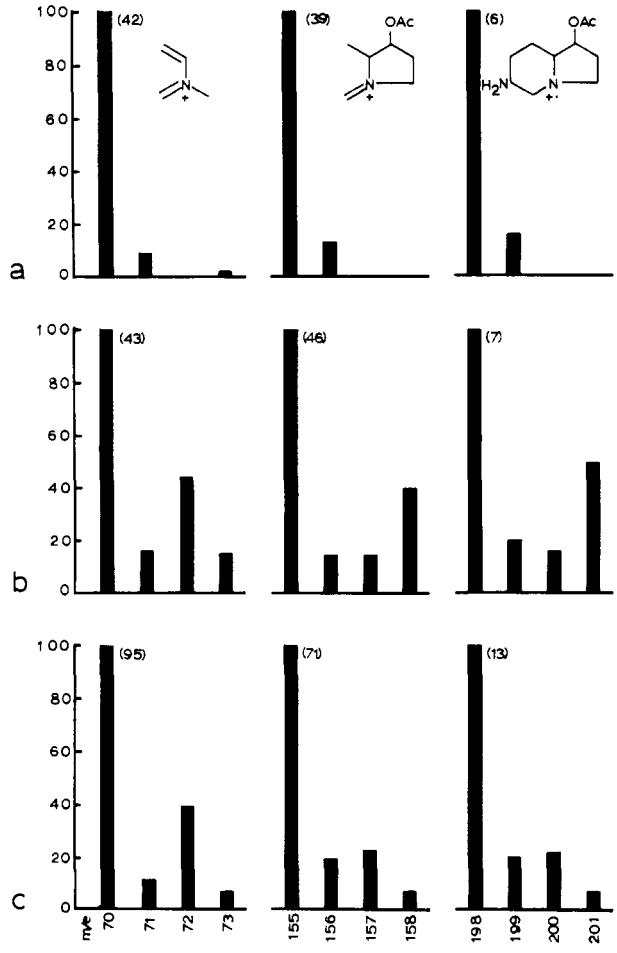


FIGURE 2: Comparison of the mass spectra of slaframine synthesized by *R. leguminicola* from (a) unlabeled precursors, (b) *cis*-[1,3,3-^{2}H]-1-hydroxy-OHI (**5a**), and (c) *trans*-[1,3,3-^{2}H]-1-hydroxy-OHI (**5b**). Intensities are normalized relative to the undeuterated ion of each multiplet (i.e., *m/e* 70, 155, or 198). Values in parentheses are intensities relative to the peak at *m/e* 44 which was the most intense peak in each spectrum.

slaframine samples showed $C_4H_8N^+$ fragments which were primarily dideuterated (i.e., *m/e* 72); however, the *m/e* 73 fragment was significantly enhanced as well (twofold in the case of slaframine derived from trideuterio **5a**), suggesting that one hydrogen atom in the ion may sometimes be derived from the 1 position.

The distinction between the direct pathway of metabolism followed by **5a** and the indirect one followed by **5b** may not be complete. Slaframine derived from *cis*-trideuterio **5a** showed significant enhancement of the P + 2 peaks, *m/e* 200 and 157, indicating that some equilibration of **5a** with ketone **6** (and concomitant loss of deuterium from the 1 position) must be occurring. Moreover, about one-quarter of trideuterio **5b** is utilized with retention of all three deuterons. A probable explanation of the latter observation is that the deuteron lost during oxidation of **5b** to **6** is retained by the redox enzyme or cofactor and reutilized in the reduction of **6** to **5a**.

Finally, it should be noted that these studies were carried out with racemic **5a** and **5b** and that the transformations are presumed to involve only the enantiomers having the *S* configurations at position 8a, identical with the 8a configuration of slaframine itself. Nothing is known about the fate of the enantiomers having *R* configurations at position 8a.

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