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Rapid Communications

Biosynthesis of Stenusine

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Abstract: The rove beetles of the genus *Stenus* Latreille synthesize the alkaloid stenusine in their pygidial glands, which are located in the last three segments of their abdomen. It is proposed that stenusine is derived from the two amino acids, L-lysine and L-isoleucine. Feeding *S. bimaculatus* beetles with deuterium-labeled amino acids and using GC/MS analysis showed that L-lysine forms the piperidine ring of the stenusine molecule. The side chain originates from L-isoleucine and the *N*-ethyl group from acetate.

Most species of the rove beetle genus *Stenus* Latreille (Coleoptera, Staphylinidae) employ stenusine [*N*-ethyl-3-(2-methylbutyl)piperidine][1] as an escape mechanism on water surfaces. In case of danger, they lower their abdomen and emit stenusine from their pygidial glands. Stenusine lowers the surface tension of the water; this effect drives the beetle rapidly over the water. This movement is called skimming.

The *Stenus* beetles synthesize stenusine in a band of secretory cells in their pygidial glands, which are located in the last three segments of their abdomen.[2]

Stenusine belongs to the group of alkaloids biogenetically derived from amino acids.[3a] Piperidine alkaloids in particular are derived from either lysine, acetate, or mevalonate.[3b] L-Lysine and 17 other amino acids were found in the pygidial glands of *S. comma* by Kohler, hence leading to the proposal that stenusine is derived from L-lysine and L-isoleucine (Figure 1).[4,5] The latter was also found in *S. comma*. To prove this hypothesis, we performed incorporation experiments with labeled amino acids and analyzed the gland contents by GC/MS.

For feeding experiments in which the compound is not expected to be replaced by its labeled analogue entirely, it is recommendable to use deuterated precursors, because they show a decrease in GC retention times and are therefore easy to detect by GC/MS analysis.[6]

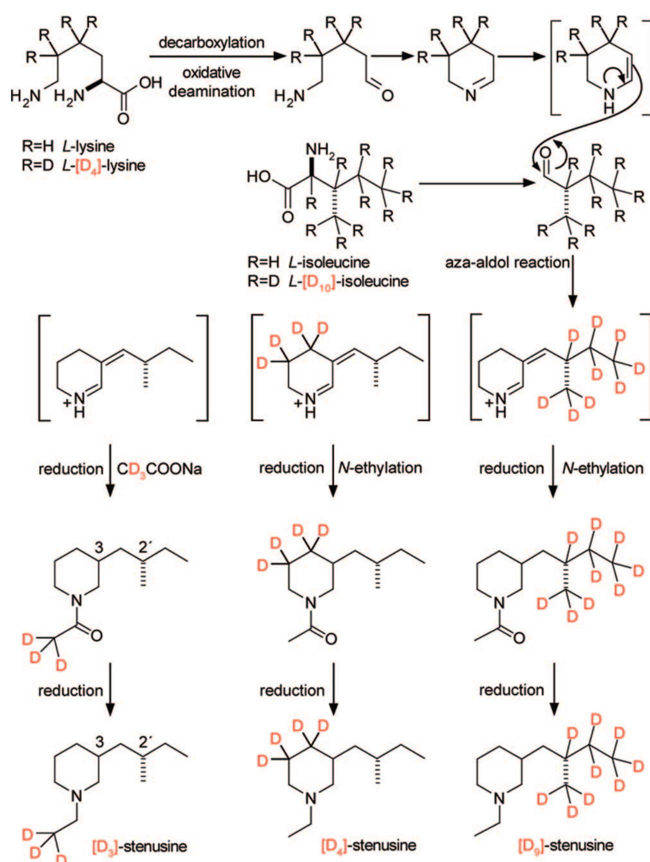

Figure 1. Putative biosynthetic pathway to stenusine in *Stenus bimaculatus*.

Depending on the amount of incorporated deuterium, the labeled compound will either be found at the beginning of the regular peak or form an extra peak before the peak of the unlabeled compound. The higher the incorporation rate, the earlier the compound elutes from the GC column; hence, an extra peak will be produced only when a high amount of deuterium is incorporated.

To facilitate recognition of the labeled stenusine in the MS, it is important to know in advance which stenusine fragments are going to show a shift in their *m*/*z* values due to the deuterium

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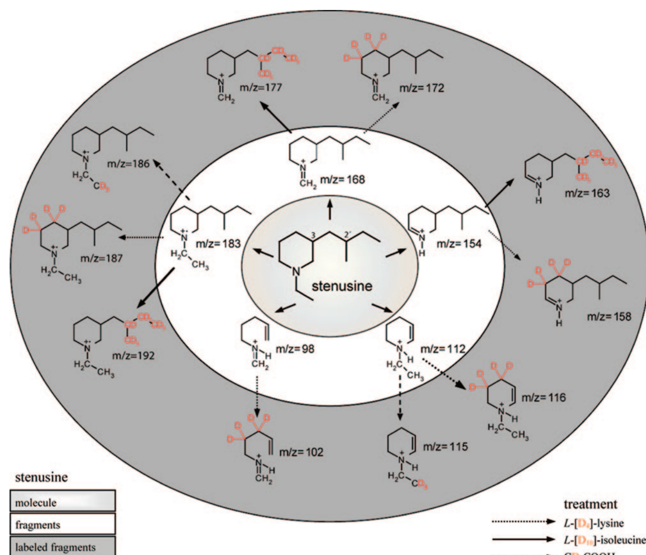

Figure 2. Fragmentation of labeled and unlabeled stenusine.

labeling. For L-[4,4,5,5-D_4]-lysine all piperidine ring fragments of stenusine are expected to shift by $\Delta m/z = 4$ (Figure 2). L-Isoleucine is supposed to form the side chain of the stenusine molecule; therefore only the $m/z = 154$, 168, and 183 fragments are likely to change.

Because of the high deuterium content, the labeled stenusine eluted earlier in the GC (~5−10 s) than its unlabeled counterpart. Mass spectra of labeled stenusine could be obtained (Figure 3).

From L-[4,4,5,5-D_4]-lysine all four deuterium atoms were incorporated by *S. bimaculatus*. It is the precursor of the piperidine ring of the stenusine molecule. Besides stenusine, norstenusine (*N*-ethyl-3-isobutylpiperidine)[7] can also be found in the majority of *Stenus* beetles. Norstenusine differs from stenusine only by one methyl group less in the side chain. The piperidine ring of norstenusine also shows a shift of $\Delta m/z = 4$ in all relevant fragments (Figure 3); hence, norstenusine is also of L-lysine origin. Its side chain might be synthesized from the amino acid L-valine.

Nine out of 10 deuterium atoms of L-[1′,1′,1′,2,3,4,4,5,5,5-D_{10}]-isoleucine were incorporated in the side chain of stenusine by *S. bimaculatus* (Figure 3).

The proposal from Poupon et al.[5] did not suggest the origin of the C_2 unit for the *N*-ethylation. Since its precursor is most likely acetate, we also conducted feeding experiments with trideuteriated sodium acetate (CD_3COONa). The fragments that were expected to show a shift in their m/z values are $m/z = 112$ and 183. They shifted by $\Delta m/z = 3$, indicating that all three deuterium atoms were incorporated (Figure 3). This proves that the *N*-ethyl group is derived from acetate.

The incorporation levels are ≤0.1%, but still detectable by GC/MS.

Experimental Section

Stenus bimaculatus beetles were kept in tanks for 1 month to get accustomed to feeding on *Drosophila melanogaster*. Then the beetles were transferred into individual Petri dishes (9 cm in diameter) that were lined with cement and 5% charcoal to prevent contamination with microorganisms and to receive a constant high humidity. The Petri dishes were cleared of dead flies and their remains regularly to prevent them from molting. Water was added when needed.

The amino acid solutions were prepared by dissolving deuterated L-isoleucine ($CD_3CD_2CD(CD_3)CD(^{15}NH_2)COOH$; Cambridge Isotopes Laboratories) and L-lysine·2HCl ($H_2NCH_2(CD_2)_2CH_2CH(NH_2)COOH \cdot 2HCl$; Cambridge Isotopes Laboratories) in sterile saline (0.9% NaCl). From both amino acids 100 μL of a 50% solution was made. The NaOAc solution (CD_3COONa; Cambridge Isotopes Laboratories) was prepared in the same way.

D. melanogaster were dipped into the solutions of the labeled amino acids and fed to the beetles. It was ensured that the fruit flies were still alive when feeding them to the beetles. Two *S. bimaculatus* beetles each were treated with L-lysine and L-isoleucine. Additionally four *S. bimaculatus* beetles fed on *D. melanogaster* that were moistened with the deuterated sodium acetate solution. All beetles were fed every second or third day with treated *D. melanogaster* for 2 weeks.

Beetles were sacrificed by freezing them at −20 °C for 1 h. Subsequently, the pygidial glands of each specimen were dissected

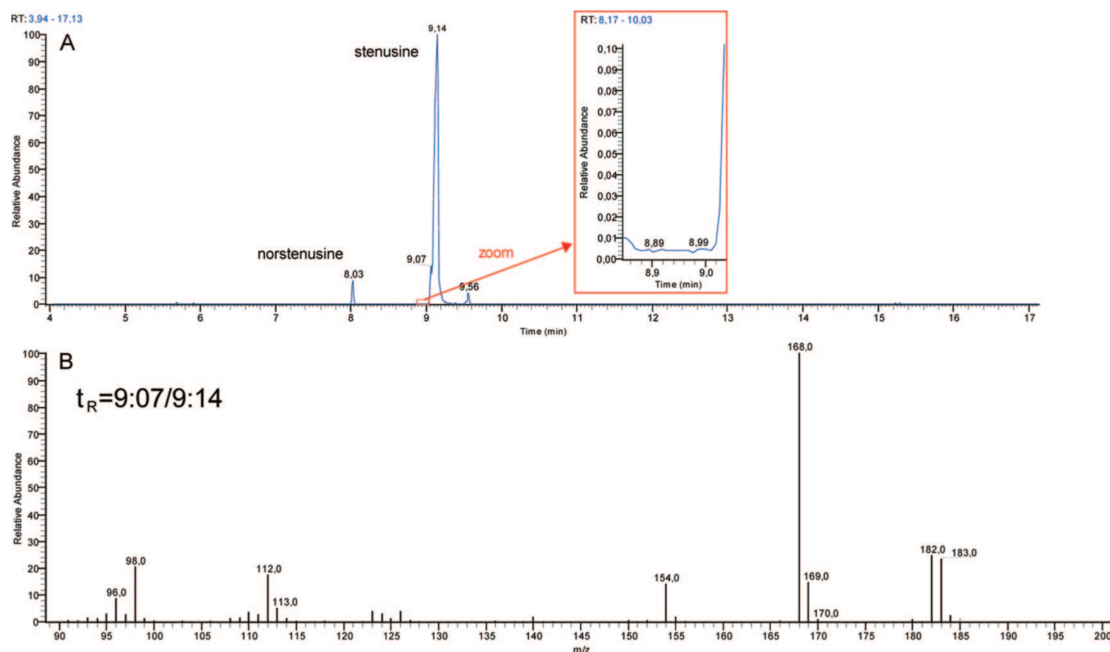

Figure 3

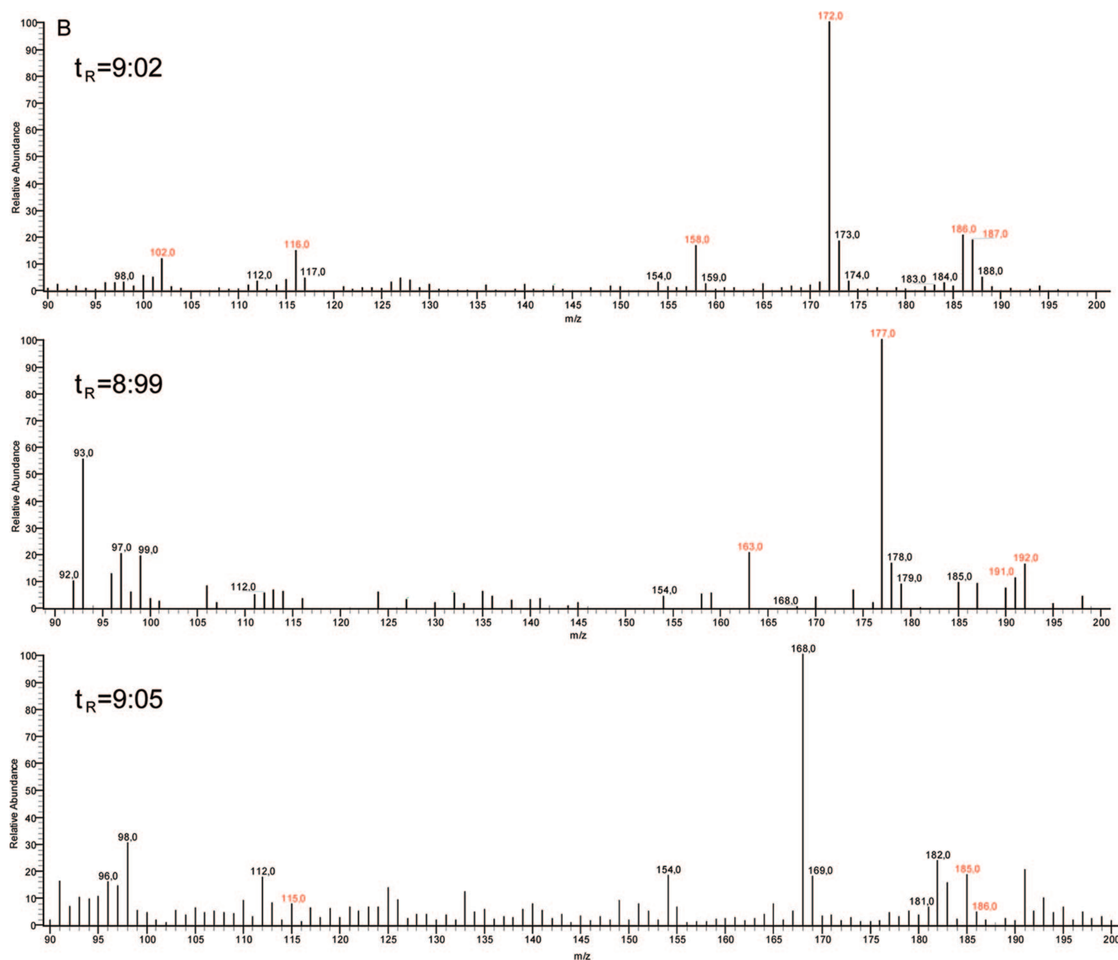

Figure 3. (A) TIC of *Stenus bimaculatus* that incorporated L-[D_{10}]-isoleucine. (B) Mass spectra top down: stenusine; incorporation of L-[D_4]-lysine, L-[D_{10}]-isoleucine, and CD_3COONa; all from *S. bimaculatus*; including retention times (t_R).

and transferred into 5 μL of EtOAc SupraSolv (Merck). Each sample was homogenized by sonication for 5 min, and 1 μL of the sample was immediately injected into the GC/MS.

GC/MS analyses were performed using a Finnigan MAT GCQ with a BPX5 column (SGE, length 25 m, diameter 0.22 mm), gas flow 1 mL/min, oven temperature program 50 °C (2 min), heating rate 10 °C/min to 275 °C.

The *S. bimaculatus* beetles that incorporated L-[D_{10}]-isoleucine showed an extra peak, which was integrated. Comparison of the area of this peak with that of pure stenusine indicated an incorporation level of ≤0.1%.

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