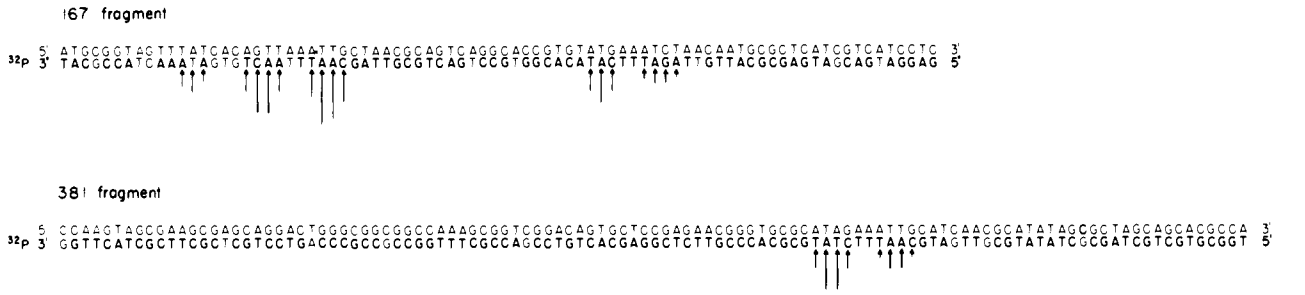
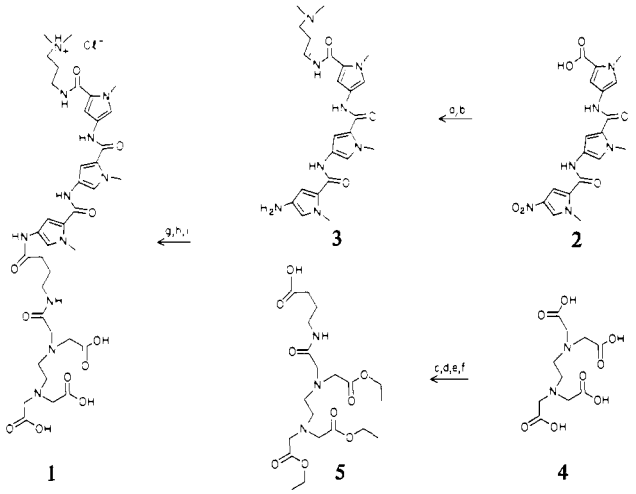


Figure 2. Arrows indicate cleavage sites on *one* strand of DNA restriction fragments by DE·Fe(II).

Scheme I



[a] $H_2N(CH_2)_3N(CH_3)_2$, HOBt, DCC. [b] H_2, Pd/C. [c] H_2SO_4, EtOH. [d] $CuCl_2$, NaOH. [e] DCC, NHS. [f] $H_2N(CH_2)_3CO_2H$, $NaHCO_3$. [g] CDI. [h] LiOH. [i] HCl.

autoradiogram of cleaved DNA fragments on the Maxam–Gilbert gel is shown in Figure 1.[20]

The MPE·Fe(II) lanes show an *even* DNA cleavage pattern, indicative of non-sequence-specific cleavage. In contrast, DE·Fe(II) shows a nonrandom pattern, with cleavage confined to highly localized sites. Comparison with the Maxam–Gilbert G lane or the bleomycin·Fe(II) lane reveals the complementary sites (A + T rich) cleaved by DE·Fe(II). Moreover, DE·Fe(II) cleaves fewer times and has higher specificity than the natural product bleomycin·Fe(II). This could be explained by the differences in the *size* of their sequence specific binding sites. In the case of bleomycin, a *two* base pair 5′–3′ GT or GC sequence is sufficient for binding and scission.[21] Although we cannot describe yet the precise details of DE binding, we believe there is a minimum three and perhaps *four* base pair recognition site composed of A and T bases. We note that predominant cleavage is centered around a 5′-ATTT-3′ site (Figure 2). The several DNA strand scissions flanking each DE·Fe(II) binding site could reflect the "reach" of the flexible tether connecting EDTA·Fe(II) to the A + T-binding pyrrolecarboxamide moieties, multiple binding modes within the preferred site, or generation of a diffusible reactive species.

With regard to future studies, the attachment of EDTA·Fe(II) to other sequence-specific DNA binding molecules such as antibiotics, polypeptides, oligonucleotides, or proteins should provide a new class of "DNA affinity cleaving molecules" and may form a primitive basis for the design and construction of artificial restriction endonucleases with defined target sequences and binding site sizes.

Acknowledgment. We are grateful to the National Institutes of Health for grant support (GM-27681).

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Biosynthesis of Swainsonine in *Rhizoctonia leguminicola*. Epimerization at the Ring Fusion

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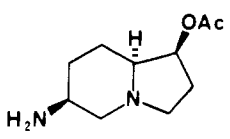
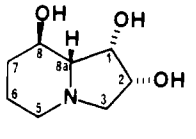
Slaframine and swainsonine[3] are alkaloidal toxins produced by *Rhizoctonia leguminicola*, a fungus that infests red clover and similar forages.[4] The biosynthesis of slaframine has been studied extensively,[4a,5] but little is known about the pathway leading to swainsonine. A preliminary study showed that, as with slaframine, pipecolic acid (and L-lysine) was incorporated into swainsonine by the fungus.[6] A later study suggested that malonate was the source of C-2 and C-3 and showed that one and two deuterium atoms of acetate-d_3 were incorporated into swainsonine and slaframine, respectively.[5a]



Slaframine



Swainsonine

A further study of the biosynthesis of swainsonine has now been undertaken employing perdeuteriopipecolic acid, which was prepared by α-methylation of pyridine-d_5 [(1) MeLi, Et_2O, room temperature; (2) refluxing benzene], oxidation of the resulting α-picoline-d_4 to the picolinic-d_4 acid·hydrochloride [(1) $KMnO_4$, H_2O, 100 °C; (2) HCl], catalytic reduction (1 atm of D_2, PtO_2, D_2O), and treatment with water to remove exchangeable deuterium atoms, to give the pipecolic-d_9 acid·hydrochloride in 29% overall yield. The mass spectrum of the product confirmed the presence of nine deuterium atoms.

(1) (a) Department of Chemistry; (b) Department of Biochemistry.
(2) (a) NSF Predoctoral Fellow 1977–1980; (b) Eastman Kodak Graduate Fellow 1980-1981.
(3) Swainsonine is also a metabolite of higher plants [*Swainsona canescens*, *Astragalus lentiginosus* (locoweed), and related species] and is thought to be responsible for their toxicity to livestock. See: Colegate, S. M.; Dorling, P. R.; Huxtable, C. R. *Aust. J. Chem.* **1979**, *32*, 2257. Molyneux, R. J.; James, L. F. *Science* (*Washington, D.C.*) **1982**, *216*, 190.
(4) (a) Guengerich, F. P.; Broquist, H. P. "Bioorganic Chemistry"; van Tamelen, E. E. Ed., Academic Press: New York, 1978, Vol. 2, Chapter 4. (b) Schneider, M. J.; Ungemach, F. S.; Harris, T. M. *Tetrahedron*, in press.
(5) (a) Clevenstine, E. C.; Broquist, H. P.; Harris, T. M. *Biochemistry* **1979**, *18*, 3658. (b) Clevenstine, E. C.; Walter, P.; Harris, T. M.; Broquist, H. P. *Ibid.* **1979**, *18*, 3663.
(6) Guengerich, F. P.; DiMari, S. J.; Broquist, H. P. *J. Am. Chem. Soc.* **1973**, *95*, 2055.

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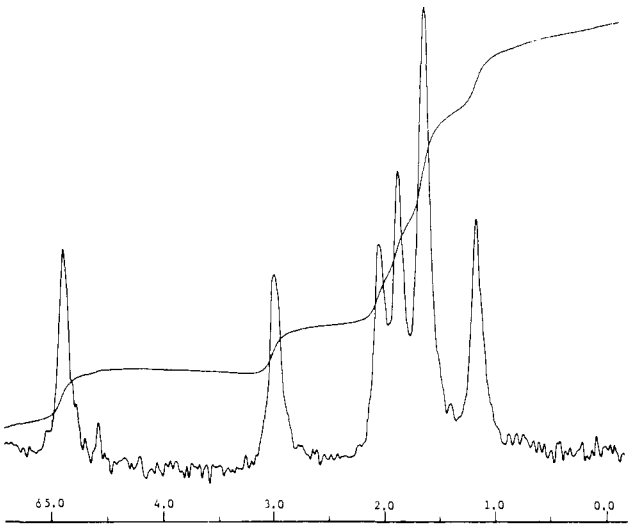

Figure 1. ^2H NMR[9] of swainsonine triacetate isolated from feeding pipecolic-d_9 acid·hydrochloride.

The pipecolic-d_9 acid·hydrochloride (0.21 mM) was fed to nine 6-day-old mycelial mats.[7] After 6 days, the alkaloids were isolated and acetylated,[4b] giving 8.7 mg of *N*-acetylslaframine and 20.4 mg of swainsonine triacetate. Mass spectrometry showed that the major isotopically labeled species in the alkaloids was d_7 (*N*-acetylslaframine, d_0:d_5:d_6:d_7 = 100:1.3:7.3:19.8, d_1–d_4 < 1.0; swainsonine triacetate, d_0:d_6:d_7 = 100:1.7:8.1, d_1–d_5 < 1.0),[8] indicating a loss of two deuterium atoms from pipecolate-d_9 in both pathways. Each alkaloid would have lost one deuterium atom during introduction of its respective substituent in the pipecolate-derived ring (slaframine, C-6 amino group; swainsonine, C-8 hydroxyl group). Identification of the site from which the second deuterium atom was lost in each case was enabled by ^2H NMR.[9] ^2H NMR of each acetylated alkaloid confirmed the presence of seven deuterium atoms. As ^1H and ^2H chemical shifts (in ppm) are the same, a ^1H signal that is not represented in the ^2H NMR spectrum can be assigned as a site from which deuterium has been lost in the biosynthesis. In the case of slaframine, the second deuterium atom lost is from the C-6 position (C-5 of pipecolate), supporting a ketonic intermediate in the biosynthesis. With swainsonine triacetate, however, the ^2H NMR spectrum (Figure 1) showed retention of deuterium at C-8 (δ 4.96). The second deuterium atom appeared to have been lost from the C-8a position of swainsonine (C-2 of pipecolate); however, as H-8a (δ 2.18) and H-7_{eq} (δ 2.0) in swainsonine triacetate have similar chemical shifts, additional supporting evidence was sought.

Confirmation of this result was obtained from a feeding experiment with [2-^2H]pipecolate. Deuterium was introduced at the 2-position of pipecolic acid by the method of Upson and Hruby.[10] The [2-^2H]pipecolic acid·hydrochloride (2.8 mM) was fed, along with [R-^3H]pipecolate (117 μCi, 0.832 μCi/mmol), to two 5-day-old mycelial mats, and after 2 days the acetylated alkaloids were isolated. Tritium was incorporated into swainsonine triacetate (0.309 μCi/mmol), but mass spectra showed no incorporation of deuterium. In contrast, the *N*-acetylslaframine contained both tritium (0.485 μCi/mmol) and deuterium (MS, d_0:d_1 = 100:124).[8] It can thus be concluded that the hydrogen atom at C-2 of pipecolate is retained in the formation of slaframine but lost in the formation of swainsonine.

In the swainsonine biosynthesis, the hydrogen atom lost from C-2 of pipecolate can be replaced by deuterium donated from

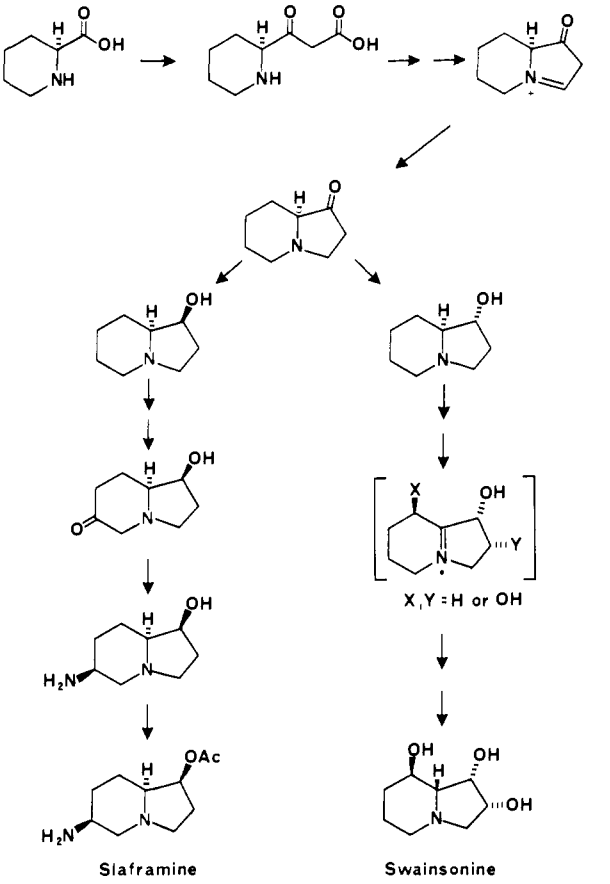

Figure 2. Proposed biosynthetic pathway for *R. leguminicola* alkaloids.

[1,1-$^2H_2$]ethanol.[11] Six-day-old mats were incubated with [1,1-$^2H_2$]ethanol (50 mM) and pipecolic acid·hydrochloride (0.2 mM) for an additional 7 days. The mass spectrum of the swainsonine triacetate showed incorporation of deuterium (d_0:d_1 = 100:3.1)[8] and the ^2H NMR spectrum[9] indicated deuterium was present at C-8a.[12] It is likely that the deuterium was introduced via a [4-^2H]nicotinamide coenzyme formed by oxidation of the ethanol to acetic acid.

During swainsonine biosynthesis, the net process at C-8a is one of epimerization; slaframine has an *S* configuration at that site while swainsonine is *R*. A reasonable proposal (Figure 2) that accounts for the stereochemical differences between the two alkaloids involves a common pathway in which L-pipecolate is coupled with malonate and the resulting pipecolylacetate undergoes reduction and cyclization to give (*S*)-1-oxooctahydroindolizine. The keto group is then reduced from both the α and β faces to lead ultimately to slaframine and swainsonine, respectively. In the formation of swainsonine, oxidation at C-8a must be postulated to occur, possibly to give an iminium ion, with subsequent reduction from the β face in order to account for the *R* configuration of the alkaloid. The timing of oxidation and reduction at C-8a relative to introduction of hydroxyl groups at C-2 and C-8 is presently being investigated.

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(7) Guengerich, F. P.; Broquist, H. P. *Biochemistry* **1973**, *12*, 4270.
(8) Mass spectra were obtained in the chemical ionization mode with ammonia as the reagent gas. Relative abundances were derived from peak intensities by correction for natural abundance ^{13}C contributions.
(9) ^2H NMR spectra were obtained at 92.2 MHz on the Carnegie-Mellon spectrometer operated in the rapid scan correlation mode with homonuclear lock on the solvent ($CDCl_3$).
(10) Upson, D. A.; Hruby, V. J. *J. Org. Chem.* **1977**, *42*, 2329.
(11) Prepared by reduction of acetyl chloride with $LiAl^2H_4$.
(12) Isotope was also incorporated at C-1, C-3, and C-5. These results are consistent with previous proposals for the biosynthesis of slaframine via 1-oxooctahydroindolizine and will be described in more detail in a subsequent paper.