Loco Intoxication: Indolizidine Alkaloids of Spotted Locoweed (*Astragalus lentiginosus*)

Abstract. *The indolizidine alkaloids swainsonine and swainsonine* N*-oxide have been isolated and identified as constituents of spotted locoweed. The inhibition of lysosomal* α*-mannosidase by these alkaloids suggests that they are the causative agents of locoism in range animals.*

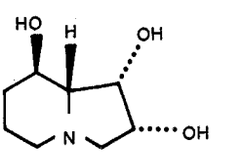
The ingestion by range animals of certain species of locoweeds (*Astragalus* and *Oxytropis* spp.), which are widely distributed in the western United States, results in a chronic neurological disease known as locoism (*1–3*). Animals poisoned by locoweed exhibit depression, a staggering gait, muscular incoordination, and a rough hair coat; they may have difficulty in eating or drinking and frequently become solitary. In addition, affected animals become nervous, especially when stressed or startled, and may attempt to leap over small or imaginary objects, walk into obstructions, or rear and fall backward (*4–6*). The condition is habit-forming, with poisoned animals seeking out the intoxicating plant. Other adverse effects are emaciation, abortion, and birth defects.

The symptoms and etiology of the disease are well defined (*7–9*). However, even though chemical investigations of various locoweeds have resulted in extracts capable of inducing the effect when fed to animals (*10, 11*), the compound responsible has never been fully identified (*11*).

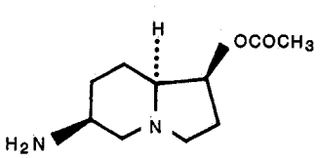
Locoism is characterized by cytoplasmic vacuolation of cells in the renal tubules and of Purkinje cells and other neurons of the central nervous system (*12*). These changes, and the neurological effects of locoweed consumption, are remarkably similar to those produced by ingestion of Darling pea (*Swainsona* spp.) (*7, 8, 13*) and to mannosidosis, a disease of humans and Angus cattle (*14*). In mannosidosis, a genetic insufficiency of acid α-mannosidase causes vacuolation of tissue cells as a result of storage of mannose-rich oligosaccharides (*15*). The indolizidine alkaloid swainsonine (Fig. 1), which has been isolated from *Swainsona canescens*, is an inhibitor of α-mannosidase (*16*). We now report the isolation and identification of swainsonine and swainsonine *N*-oxide from spotted locoweed (*Astralagus lentiginosus*).

Dried, milled, aboveground parts of *A. lentiginosus*, collected in Utah and identified at the Intermountain Herbarium, were extracted with hot acetone to yield, on cooling, a white, waxy solid (1.7 percent). The major portion of this material consisted of (+)-pinitol, a common constituent of leguminous plants (*17*). Examination of the crude extract by thin-layer chromatography showed no aliphatic nitro compounds; these compounds are responsible for the acute toxicity of certain *Astragalus* species commonly known as poison vetches (*18*). Purification of the water-soluble portion of the solid material by chromatography on AG50W-X8 cation-exchange resin gave 0.007 percent yield of a basic material, which by thin-layer chromatography (*19*) showed the presence of two compounds, both reacting positively to alkaloid-specific Dragendorff's reagent. The two alkaloids were separated by their differential solubility in chloroform. The soluble material, which crystallized as white needles (0.003 percent) was shown by thin-layer chromatography, mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy to be identical with swainsonine (*16, 20*).

The chloroform-insoluble alkaloid, which was less mobile than swainsonine on thin-layer chromatography, crystallized from acetone as hygroscopic, pale yellow prisms (0.001 percent) (*20*). Combustion analysis indicated a molecular formula of $C_8H_{15}NO_4$. The electron-impact mass spectrometry of this compound, determined at a probe temperature of 180°C, was virtually identical with that of swainsonine, but the isobutane chemical ionization spectrum, determined at 140°C, exhibited a molecular ion at a mass-to-charge ratio of 190 (base peak), with major fragments at 174 (22 percent), 172 (67 percent), 154 (44 percent), and 136 (13 percent). The peaks correspond to loss of oxygen and sequential loss of three molecules of water from the molecular ion. These results indicated that the alkaloid was swainsonine *N*-oxide, the lack of a parent ion at higher probe temperatures being due to thermal deoxygenation (*21*). The ^1H NMR spectrum, determined in ^2H_2O solution, was more complex than that of swainsonine, but showed no evidence of unsaturation, with no signals below δ 4.5. The ^{13}C NMR spectrum measured in ^2H_2O with acetonitrile as a reference, showed eight signals at δ 21.7, 33.2, 65.2, 65.5, 71.4, 73.0, 78.4, and 79.4 ppm. Insufficient material was available to obtain the partially decoupled spectrum (*22*).



Swainsonine



Slaframine

Fig. 1. Structure of the indolizidine alkaloids swainsonine and slaframine.

On treatment with zinc dust in 2*N* hydrochloric acid, the *N*-oxide underwent slow, partial reduction to swainsonine; with sodium borohydride in methanol, the reduction was rapid and complete. Conversely, oxidation of swainsonine with hydrogen peroxide in ethanol yielded a product whose mass spectrum and other properties were identical to those of natural swainsonine *N*-oxide. The *N*-oxide was as effective as swainsonine for inhibiting hydrolysis of 4-methylumbelliferyl-α-D-mannopyranoside by either mouse liver homogenate or jack-bean α-mannosidase in citric acid–sodium hydroxide buffer (*p*H 4), as shown by comparison with the development of the bright blue ultraviolet fluorescence in uninhibited control samples (*16*). The oxidation of swainsonine to the *N*-oxide form therefore does not appear to affect its ability to inhibit the enzyme.

The isolation of swainsonine from spotted locoweed and Darling pea appears to represent the first reported examples of the occurrence of an indolizidine alkaloid in the Leguminosae family. In addition, swainsonine *N*-oxide is established as a naturally occurring indolizidine *N*-oxide alkaloid. The genera *Swainsona* and *Astragalus* have been classified in the adjacent tribes Coluteae and Astragaleae, respectively, of the subfamily Lotoideae (*23*). The genus *Oxytropis* is also a member of the Astragaleae, and preliminary examination of extracts of white locoweed (*Oxytropis sericea*) by thin-layer chromatography has indicated the presence of swainsonine and its *N*-oxide in this plant also.

In contrast to many other classes of alkaloids, simple indolizidine alkaloids do not appear to be widespread in nature. The only other indolizidine alkaloid with known physiological activity in animals is the fungal metabolite slaframine

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(Fig. 1) from *Rhizoctonia leguminicola*, which is responsible for producing excessive salivation in cattle (*24*). The occurrence of indolizidine alkaloids in the Leguminosae emphasizes their structural affinity with the pyrrolizidine and quinolizidine alkaloids (*25*). Quinolizidine alkaloids are quite widespread in legumes, and it is possible that indolizidine alkaloids may also occur more frequently in this plant family than has been previously demonstrated.

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References and Notes

1. J. M. Kingsbury, *Poisonous Plants of the United States and Canada* (Prentice-Hall, Englewood Cliffs, N.J., 1964), p. 305.
2. *Science* **13**, 176 (1889).
3. Loco, a word of Spanish origin meaning "crazy" or "mad," was applied by the early Spaniards to the disease common among horses, cattle, and sheep of the Southwest. The word is now well established in colloquial English.
4. C. D. Marsh, *U.S. Dep. Agric. Bull. No. 1245* (1924), p. 16.
5. L. F. James, W. J. Hartley, K. R. Van Kampen, *J. Am. Vet. Med. Assoc.* **178**, 146 (1981).
6. L. F. James, J. L. Shupe, W. Binns, R. F. Keeler, *Am. J. Vet. Res.* **28**, 1379 (1967).
7. C. D. Marsh, *U.S. Dep. Agric. Bur. Anim. Ind. Bull. No. 112* (1909).
8. A. C. Crawford, *U.S. Dep. Agric. Bur. Plant Ind. Bull. No. 129* (1908). A loss of 25,000 cattle due to locoweed poisoning in one area of 35 by 120 miles in southwestern Kansas in 1883 is cited in this bulletin.
9. F. P. Mathews, *Tex. Agric. Exp. Stn. Bull. No. 456* (1932).
10. J. F. Couch, *J. Pharmacol. Exp. Ther.* **36**, 55 (1929).
11. G. S. Fraps and E. C. Carlyle, *Tex. Agric. Exp. Stn. Bull. No. 537* (1936).
12. K. R. Van Kampen and L. F. James, *Pathol. Vet.* **6**, 413 (1969); *ibid.* **7**, 503 (1970).
13. L. F. James, K. R. Van Kampen, W. J. Hartley, *ibid.* **7**, 116 (1970); W. J. Hartley, in *Effects of Poisonous Plants on Livestock*, R. F. Keeler, K. R. Van Kampen, L. F. James, Eds. (Academic Press, New York, 1978), p. 363.
14. P. R. Dorling, C. R. Huxtable, P. Vogel, *Neuropathol. Appl. Neurobiol.* **4**, 285 (1978).
15. P. A. Ockerman, in *Lysosomes and Storage Diseases*, H. G. Hers and F. Van Hoof, Eds. (Academic Press, New York, 1973).
16. S. M. Colegate, P. R. Dorling, C. R. Huxtable, *Aust. J. Chem.* **32**, 2257 (1979); *Biochem. J.* **191**, 649 (1980).
17. J. E. Courtois and F. Percheron, in *Chemotaxonomy of the Leguminosae*, J. B. Harborne, D. Boulter, B. L. Turner, Eds. (Academic Press, New York, 1971), p. 215.
18. W. Majak and R. J. Bose, *Phytochemistry* **13**, 1005 (1974); M. C. Williams and R. J. Barneby, *Brittonia* **29**, 310 (1977).
19. Thin-layer chromatography was performed on aluminum-backed silica gel 60 0.25-mm coated plates, developed with a mixture of chloroform, methanol, and 17 percent ammonium hydroxide (82.5:15.5:2 or 70:26:4).
20. The soluble material had a melting point of 144° to 146°C (decomposition) and values of optical rotation of $[\alpha]^{25}_{589}$, −84.0°; $[\alpha]^{25}_{578}$, −87.7°; $[\alpha]^{25}_{546}$, −99.2°; and $[\alpha]^{25}_{436}$, −165.4°; ethanol (*c*, 0.99). This substance when mixed with an authentic sample of swainsonine had a melting point of 144° to 146°C. The optical rotation of the authentic sample was measured as $[\alpha]^{24}_{589}$, −83.4°; $[\alpha]^{24}_{578}$, −87.2°; $[\alpha]^{24}_{546}$, −98.4°; and $[\alpha]^{24}_{436}$, −164.7°; ethanol (*c*, 0.32). The chloroform-insoluble alkaloid had a melting point of 161° to 163°C (decomposition).
21. J. B. Bremer and L. Van Thuc, *Aust. J. Chem.* **33**, 379 (1980); J. D. Phillipson and S. S. Handa, *Phytochemistry* **14**, 999 (1975). The mass spectrum of the pyrrolizidine alkaloid retronecine *N*-oxide exhibited a parent ion with intensity of 0.7 percent at 180°C.
22. The ^{13}C NMR spectrum of swainsonine, determined under the same conditions, exhibited four methylene (δ 25.3, 34.6, 54.0, and 62.6 ppm) and four methine (δ 68.3, 71.3, 71.8, and 75.1 ppm) carbon resonances.
23. V. H. Heywood, in *Chemotaxonomy of the Leguminosae*, J. B. Harborne, D. Boulter, B. L. Turner, Eds. (Academic Press, New York, 1971), p. 1.
24. S. D. Aust, H. P. Broquist, K. L. Rinehart, *J. Am. Chem. Soc.* **88**, 2879 (1966).
25. J. A. Mears and T. J. Mabry, in *Chemotaxonomy of the Leguminosae*, J. B. Harborne, D. Boulter, B. L. Turner, Eds. (Academic Press, New York, 1971), p. 73.
26. We thank Dr. P. R. Dorling for generously providing an authentic sample of swainsonine.

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Pregnancy Suppression by Active Immunization Against Gestation-Specific Riboflavin Carrier Protein

Abstract. *A riboflavin carrier protein isolated from chickens cross-reacts with a gestation-specific rodent carrier for riboflavin. Active immunization of female rats of proved fertility with the purified chicken carrier protein completely yet reversibly suppressed early pregnancy without impairing implantation per se. Concurrently there were no discernible adverse effects on maternal health in terms of weight gain, vitamin status, and fertility.*

The mechanism of facilitated transplacental transport and fetal accumulation of riboflavin (and other B group vitamins) in pregnant humans and other mammals (*1, 2*) is unknown, although the relative impermeability of placental membranes to the free vitamin and its coenzyme forms is well recognized (*3*). Recently, we obtained biochemical (*4*) and immunological (*5*) evidence of a gestation-specific high-affinity riboflavin carrier protein (RCP) in the rat. This RCP cross-reacts immunologically with the vitamin carrier in the chicken; in the avian system this vitamin carrier is obligatory for vitamin deposition in the developing oocyte (*6*). The rodent RCP, like its avian counterpart (*7*), is estrogen-inducible and its circulatory concentrations are modulated in concert with changing hormonal levels (*5*). Its importance in fetal development and survival was demonstrated in vivo by passive immunoneutralization which invariably led to acute fetal wastage and abrupt termination of pregnancy (*5*). These observations raised the possibility that immunoneutralization of the RCP in vivo could be exploited as a potentially useful approach to fertility regulation. We now report that active immunization of fertile rats with avian RCP repeatedly terminates early pregnancy with no discernible adverse effects on maternal health in terms of growth, vitamin status, cyclicity, and fecundity.

Part of our evidence for the obligatory participation of RCP in transplacental vitamin transport stems from experiments (*8*) wherein pregnant rats, injected with [^{14}C]riboflavin, were administered a potent and specific antiserum to purified

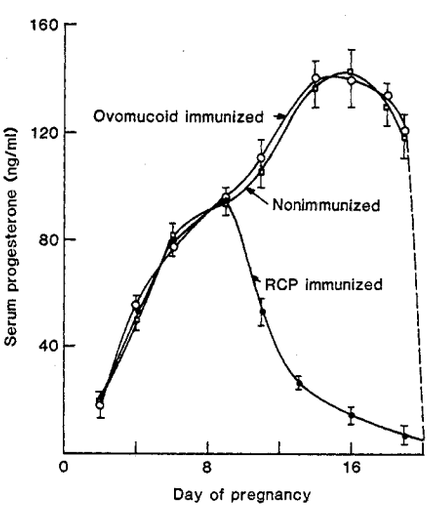

Fig. 1. Effects of active immunization against riboflavin carrier protein and ovomucoid on progesterone concentrations during gestation in rats. The immunization protocol was as described in Table 1. Estrous cycles were evaluated by microscopic examination of vaginal smears. Estrous females were mated with fertile adult males, and the day on which sperms were detected in the vaginal smears was taken as day 1 of pregnancy. Serum prepared from blood withdrawn by cardiac puncture was taken for progesterone estimation by radioimmunoassay (*13*). Serum was deproteinized with methanol and the steroid fraction was extracted with ether. The ether was evaporated off and the steroids were dissolved in buffer (0.01*M* phosphate buffered saline, *p*H 7.4, containing 0.1 percent gelatin). Portions of this were incubated with 1:2000 diluted specific antiserum to progesterone along with ≃ 8000 count/min of [^3H]progesterone (101 Ci/mmole) and then incubated for 3 hours at 0°C; the unbound progesterone was removed by dextran-coated charcoal. The radioactivity of the antibody-bound progesterone was counted.

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