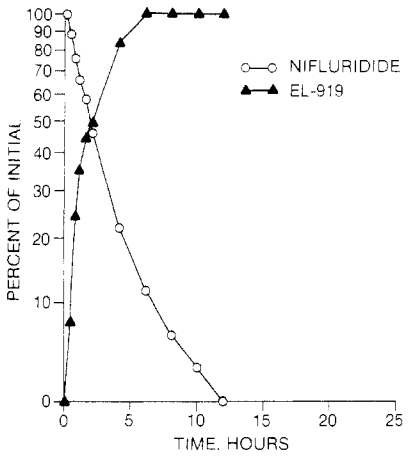


Figure 4. Rate of reaction of nifluridide at pH 9.0 with the formation of EL-919.

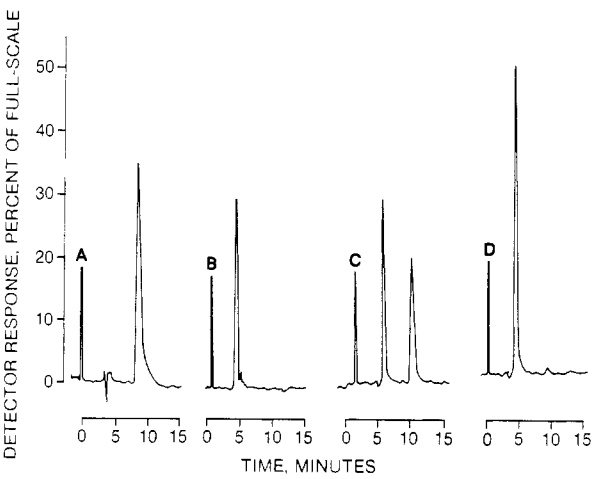


Figure 5. Chromatograms demonstrating the dissipation of nifluridide and the formation of EL-919 at pH 9.0: (A) nifluridide standard, 0.8 μg; (B) EL-919 standard, 0.8 μg; (C) reaction solution at 2 h (45.8% nifluridide, 49.1% EL-919); (D) reaction solution at 10 h (2.9% nifluridide, 105.7% EL-919).

balance was obtained by adding together the concentrations of nifluridide and EL-919 (as its nifluridide molecular weight equivalent).

RESULTS AND DISCUSSION

The rapid conversion of nifluridide to EL-919 proceeds as indicated in Figure 1. The rates of disappearance and the corresponding formation of EL-919 are plotted on a semilogarithmic scale in Figures 2–4. The cyclization proceeded via a first-order reaction with half-lives of 15.5, 3.5, and 2.0 h for EL-468 at pH 5.0, 7.0, and 9.0, respectively. The total amount of nifluridide and EL-919 present in the final samples analyzed provided a good material balance (80–100%) compared to the initial amount of chemical in the aqueous solutions (Figures 2–4). No other hydrolysis products were observed.

On the basis of these results, nifluridide would likely dissipate very rapidly in natural water or in the presence of soil moisture. Thus, the parent compound would not be expected to persist in the environment.

Chromatograms demonstrating the determination of both compounds in the aqueous solutions are shown in Figure 5. The direct injection of the solutions into the reverse-phase HPLC system at specified intervals, which were programmed into the WISP, provided an automated rate study by eliminating the need to extract the buffered solutions with a suitable solvent, concentrate the sample extract, and redissolve the residue in a suitable solvent for injection into the HPLC. It is probable that this direct injection technique could also be applied to many other compounds of environmental interest that are highly labile in aqueous solution.

LITERATURE CITED

Day, E. W.; Dorulla, G. K.; West, S. D.; Decker, O. D., presented at the 183rd National Meeting of the American Chemical Society, Las Vegas, NV, March 1982.

Lilly Research Laboratories "Technical Report on EL-468 Experimental Insecticide"; Lilly Research Laboratories: Indianapolis, IN, 1981.

Sheldon D. West*
Edgar W. Day, Jr.

Agricultural Analytical Chemistry
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

Received for review December 18, 1981. Accepted April 23, 1982.

Reinvestigation of the Alkaloids of *Lupinus sericeus* Pursh. Identification of a New Natural Product, 10,17-Dioxo-β-isosparteine

In addition to two previously reported *Lupinus sericeus* constituents, sparteine and β-isosparteine, 10,17-dioxo-β-isosparteine, a new natural product, and 17-oxosparteine, 10-oxo-β-isosparteine, and nuttalline (4β-hydroxylupanine) were also found in the aerial parts of this toxic range plant. Anagyrine, a suspected teratogenic constituent of this lupine for cattle, was not detected in the sample of this species under investigation. (−)-Sparteine exhibited the most potent acute toxic response for mice among the *L. sericeus* alkaloidal constituents tested.

Lupinus sericeus Pursh (silky lupine), a range plant indigenous to the Rocky Mountain states, has been cited as being one of the six most toxic lupines for sheep in the United States and Canada (Kingsbury, 1964; Clarke, 1970).

0021-8561/82/1430-0796$01.25/0

Ingestion of large quantities of this plant may result in respiratory disturbances, coma, and death (Clarke, 1970). *L. sericeus* has also been demonstrated as a cause of the congenital disorder crooked calf disease (Shupe et al., 1967a). The quinolizidine alkaloid constituents of this lupine, which are thought to be responsible for its toxic effects, have recently been reviewed (Smolenski et al., 1981).

Work was undertaken in the present investigation on the identification and acute toxicity determination in mice of the alkaloids of *L. sericeus*, collected in Utah in June 1964, for two reasons. First, it is known that the quinolizidine alkaloid constituents of a particular lupine may vary qualitatively with factors such as time and place of collection (Kinghorn et al., 1980). Therefore, it was of interest to determine if the alkaloids of the plant material examined in this study differed phytochemically from those identified in this species by other workers. Second, in an attempt to evaluate whether or not *L. sericeus* really deserves a reputation of being one of the most toxic North American lupines, LD_{50} determinations in mice were carried out on the major alkaloids isolated, as well as on the total alkaloidal extract from the plant.

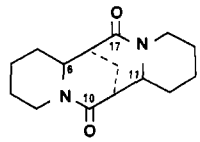
EXPERIMENTAL SECTION

Apparatus. GC/MS (70 eV) was performed on a Varian MAT 112 S mass spectrometer, equipped with a Varian 166 data system, linked to a Varian 1440 gas chromatograph. The following operating conditions were used: 2 m × 3 mm glass column, packed with 3% OV-17 on Gas-Chrom Q, mesh size 100–120. The injector temperature was 260 °C, and the oven temperature was programmed from 180 to 310 °C at 4 °C/min. The GC/MS interface temperature was 240 °C, and the helium flow rate was 18 mL/min. Optical rotations were determined on a Perkin-Elmer 241 polarimeter.

Plant Material. The above-ground parts of *L. sericeus* Pursh, collected in Utah in June 1964 were supplied by the Developmental Therapeutics Program (Natural Products Branch) of the National Cancer Institute, formerly the Cancer Chemotherapy National Service Center, Bethesda, MD. A voucher specimen representing this collection is deposited at the Herbarium of the National Arboretum, Washington, DC.

Extraction of Alkaloids. Dried, milled plant material (575 g) was exhaustively extracted with 80% ethanol, and the resulting extract (26.6 g) was basified with 28% NH_4OH, dried on a steam bath, and refluxed for 1 h with $CHCl_3$ (2 × 150 mL). Filtrates were combined and partitioned with 1% w/v HCl (100 mL). The acidic layer was adjusted to pH 8.5 with 28% NH_4OH and extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ layers were combined and concentrated in vacuo to yield 7.23 g of crude alkaloidal fraction, representing 1.26% w/w of the plant material.

Identification of Alkaloids. When the crude alkaloidal fraction (10 mg) was diluted with CH_2Cl_2 to produce a 10 mg/mL solution and analyzed by GC/MS, six quinolizidine alkaloids were identified (Table I). Sparteine, β-isosparteine, 17-oxosparteine, and nuttalline were identified on the basis of direct comparison (t_r, MS) with authentic samples and appropriate stereoisomers in a similar manner to previous studies (Kinghorn et al., 1980, 1982). 10-Oxo-β-isosparteine, t_r = 16.8 min, exhibited a mass spectrum identical with that of 17-oxosparteine (Schumann et al., 1968). 10,17-Dioxo-β-isosparteine (1), t_r = 21.5 min, exhibited the following mass spectrum: m/z (rel intensity) 262 (M^+, 26), 234 (6), 206 (6), 152 (15), 151 (22), 150 (100), 124 (27), 111 (30), 110 (27), 97 (17), 84 (66), 55 (32), and 41 (38).



(6R:11R)

1

Isolation of Major Alkaloids. A portion (2 g) of the crude alkaloidal fraction of *L. sericeus* was subjected to low-pressure column chromatography over a Lichoprep Si 60 column (40–63 μm, Merck, Darmstadt, West Germany), eluted with $CHCl_3$–MeOH–28% NH_4OH (85:15:2). Eluates were monitored by TLC and 30 10-mL fractions were obtained. Fractions 3 and 4 were combined to yield a residue which was resolved by preparative TLC into (−)-17-oxosparteine [4.3 mg, 0.06%; oil; $[\alpha]_D^{25}$ −10.5° (*c* 0.4, EtOH)] and (−)-10-oxo-β-isosparteine [15 mg, 0.20%; oil; $[\alpha]_D^{25}$ −4.8° (*c* 0.15, EtOH)]. Fractions 20–30 were combined to yield (−)-β-isosparteine [49 mg, 0.68%; resin; $[\alpha]_D^{25}$ −9.5° (*c* 0.30, EtOH)]. A second portion of the crude alkaloidal fraction (1 g) was subjected to low-pressure liquid chromatography over silica gel 60 using mixtures of C_6H_6–$CHCl_3$ and $CHCl_3$–MeOH–28% NH_4OH and preparative TLC to yield (−)-sparteine [21 mg, 0.29%; oil; $[\alpha]_D^{25}$ −17° (*c* 0.53, EtOH)].

Partial Synthesis of 10-Oxo-β-isosparteine and 10,17-Dioxo-β-isosparteine (1). A suspension of (−)-β-isosparteine (30 mg) in 5 mL of H_2O was treated with a mixture of $K_3Fe(CN)_6$ (110 mg) and KOH (15 mg) in H_2O (3 mL) according to a published procedure (Moore and Marion, 1953). Analysis of a 1% w/v solution of the reaction products in CH_2Cl_2 by GC/MS showed them to consist of 10-oxo-β-isosparteine and 10,17-dioxo-β-isosparteine (1), in a ratio of about 10.5:1. These reaction products exhibited identical retention and mass spectral data with those reported above for 10-oxo-β-isosparteine and 10,17-dioxo-β-isosparteine (1) from *L. sericeus*.

Acute Toxicity of *L. sericeus* Alkaloids in Mice. Male Swiss Webster mice, weighing 15–25 g, were injected intraperitoneally with the crude alkaloidal fraction of *L. sericeus*, as well as with (−)-sparteine, (−)-β-isosparteine, (−)-17-oxosparteine, and (−)-10-oxo-β-isosparteine. An additional quantity of (−)-17-oxosparteine was obtained for this purpose by reaction of authentic (−)-sparteine with alkaline ferricyanide. Compounds were suspended in 0.5% aqueous Tween 80. Five doses for each compound were administered to groups of six mice, with doses varying by a log factor of 2. LD_{50} and standard deviation data (Table I) were calculated by using the method of Miller and Tainter (1944).

RESULTS AND DISCUSSION

Combined GC/MS has been used to identify quinolizidine alkaloids from *Lupinus* species previously, without the need for solute derivatization (Anderson and Martin, 1976; Kinghorn et al., 1980). Six alkaloids were identified from a sample of *L. sericeus*, collected in Utah in June 1964 by using this technique (Table I). Only two of the compounds detected, sparteine and β-isosparteine, have been obtained before from this taxon (Smolenski et al., 1981). Of the remaining alkaloids identified, 17-oxosparteine and nuttalline (4β-hydroxylupanine) appear to be common quinolizidine constituents of North American lupines (Kinghorn et al., 1980), although 10-oxo-β-isosparteine has been isolated only once previously from a

Table I. Alkaloidal Constituents of *L. sericeus* Pursh and Their Acute Toxicity for Mice

constituent	GLC t_r, min[a]	GLC area, %[b]	acute toxicity[c,d] LD_{50}, mg/kg[e,f]	SD
(−)-sparteine	5.7	23.2	47.5	1.1
(−)-β-isosparteine	6.6	54.1	92.5	0.6
(−)-17-oxosparteine	14.0	4.4	468.0	7.1
(−)-10-oxo-β-isosparteine	16.8	16.1	>600	[g]
nuttalline[h]	17.9	0.9	[g]	[g]
10,17-dioxo-β-isosparteine[h]	21.5	1.3	[g]	[g]

[a] 3% OV-17 column. [b] Yield of total alkaloidal extract, 1.26% w/w (of dried plant material). [c] Determined on male Swiss Webster mice. [d] Test compounds administered in 0.5% aqueous Tween 80, injected ip. [e] Calculated by the method of Miller and Tainter (1944); data read 48 h after compound administration. [f] Total alkaloidal extract, LD_{50} = 76.0 ± 3.9 mg/kg. [g] Not determined. [h] Optical sign not determined since constituent present in too small a quantity for isolation.

legume, namely, *Lupinus nuttallii* (Mears and Mabry, 1971). The structure of 10,17-dioxo-β-isosparteine (1), a minor alkaloidal constituent of the *L. sericeus* sample investigated, was confirmed by partial synthesis from β-isosparteine with alkaline ferricyanide. This reagent, used previously to oxidize β-isosparteine to 10-oxo-β-isosparteine (Moore and Marion, 1953), is also known to produce 1 from this same starting compound (Carmack et al., 1967). In the mass spectrum of 1, fragment peaks corresponding to successive losses of CO from the parent ion were observed at *m/z* 234 and 206. Such M–CO fragments are characteristic of the mass spectra of both 10- and 17-oxosparteines but not of lupanine (2-oxosparteine) derivatives (Anderson and Martin, 1976). While compound 1 was detected as a natural product for the first time in this study, it was produced synthetically some time ago (Bohlmann et al., 1957).

The teratogenicity of *L. sericeus* for cattle has been unequivocally established by feeding trials (Shupe et al., 1967a). Anagyrine, a pyridone quinolizidine base implicated as the teratogen in this condition (Keeler, 1973), has been reported as a constituent of the aerial parts of *L. sericeus*, collected from 11 locations in three Rocky Mountain states during the late spring to early autumn of 1972 (Keeler et al., 1976). However, anagyrine was not detected in the present study on this species. This negative result, coupled with the large variations in anagyrine levels in samples of this species reported by Davis (1982), may help explain observed differences in the incidence of fetal deformities associated with pregnant cows that are known to have foraged *L. sericeus* during susceptible periods of the gestation period for crooked calf disease (Shupe et al., 1967a,b).

(−)-Sparteine was found to be the most toxic for mice of the *L. sericeus* alkaloids tested in this study (Table I). While no extensive testing of the acute toxicity of lupine quinolizidine alkaloids appears to have been performed in experimental animals, (−)-sparteine was reported as being 95% as toxic to guinea pigs as (+)-lupanine, when injected intravenously (Couch, 1926). Since lupanine is a more common constituent of North American lupines than sparteine (Kinghorn et al., 1980), and because the yield of sparteine in the *L. sericeus* sample examined in this study was not abnormally high, the reputation of this species in being an abnormally toxic lupine for sheep seems to be without foundation. However, in view of the alkaloidal variability inherent in different samples of *L. sericeus* that has been referred to, further toxicity testing is necessary before a definitive statement of this type may be made.

ACKNOWLEDGMENT

We thank Professor T. J. Mabry, University of Texas at Austin, Austin, TX, for the donation of (−)-sparteine and 17-oxosparteine and Professor W. J. Keller, Northeast Louisiana University, Monroe, LA, for β-isosparteine perchlorate.

LITERATURE CITED

Anderson, J. N.; Martin, R. O. *J. Org. Chem.* **1976**, *41*, 3441–3444.
Bohlmann, F.; Weiser, W.; Sander, H.; Hanke, H.-G.; Winterfeldt, E. *Chem. Ber.* **1957**, *90*, 653–661.
Carmack, M.; Goldberg, S. I.; Martin, E. W. *J. Org. Chem.* **1967**, *32*, 3045–3049.
Clarke, E. C. G. In "The Alkaloids, Chemistry and Physiology"; Manske, R. H. F., Ed.; Academic Press: New York and London, 1970; Vol. 12, pp 514–589.
Couch, J. F. *J. Agric. Res. (Washington, D.C.)* **1926**, *32*, 51–67.
Davis, A. M. *J. Range Manage.* **1982**, *35*, 81–84.
Keeler, R. F. *Teratology* **1973**, *7*, 23–30.
Keeler, R. F.; Cronin, E. H.; Shupe, J. L. *J. Toxicol. Environ. Health* **1976**, *1*, 899–908.
Kinghorn, A. D.; Balandrin, M. F.; Lin, L.-J. *Phytochemistry* **1982**, in press.
Kinghorn, A. D.; Selim, M. A.; Smolenski, S. J. *Phytochemistry* **1980**, *19*, 1705–1710.
Kingsbury, J. M. "Poisonous Plants of the United States and Canada"; Prentice-Hall: Englewood Cliffs, NJ, 1964; pp 333–341.
Mears, J. A.; Mabry, T. J. In "Chemotaxonomy of the Leguminosae"; Harborne, J. B.; Boulter, D.; Turner, B. L., Eds.; Academic Press: London and New York, 1971; pp 73–178.
Miller, L. C.; Tainter, M. L. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261–264.
Moore, B. P.; Marion, L. *Can. J. Chem.* **1953**, *31*, 187–192.
Schumann, D.; Neuner-Jehle, N.; Spiteller, G. *Monatsh. Chem.* **1968**, *99*, 390–408.
Shupe, J. L.; Binns, W.; James, L. F.; Keeler, R. F. *J. Am. Vet. Med. Assoc.* **1967a**, *151*, 198–203.
Shupe, J. L.; James, L. F.; Binns, W. *J. Am. Vet. Med. Assoc.* **1967b**, *151*, 191–197.
Smolenski, S. J.; Kinghorn, A. D.; Balandrin, M. F. *Econ. Bot.* **1981**, *35*, 321–355.

In-Chull Kim
Manuel F. Balandrin
A. Douglas Kinghorn*

Department of Pharmacognosy and Pharmacology
College of Pharmacy University of Illinois at the Medical Center
Chicago, Illinois 60612

Received for review December 31, 1981. Accepted April 16, 1982. M.F.B. received the award of the S. B. Penick Memorial Fellowship (1978–1981) from the American Foundation for Pharmaceutical Education.