

Phytochemical Survey of Connecticut I: Isolation of Monocrotaline from *Crotalaria sagittalis* L. Fruit

ROBERT E. WILLETTE[▲] and LAWRENCE V. CAMMARATO

Abstract □ *Crotalaria sagittalis* L., commonly known as rattlebox, is the oldest species of its genus known to cause "crotalism," but it has not been previously investigated. In the present study, monocrotaline was isolated and identified as the major alkaloid present in the fruit. The cooccurrence of its *N*-oxide and three other pyrrolizidine alkaloids was demonstrated. Monocrotaline is the major alkaloid of several *Crotalaria* species.

Keyphrases □ Monocrotaline— isolation from *Crotalaria sagittalis* L. fruit □ *Crotalaria sagittalis* L. fruit— isolation of monocrotaline □ Phytochemical survey of Connecticut— monocrotaline isolation from *Crotalaria sagittalis*

Crotalaria sagittalis L. appears to be the first of its genus reported as a toxic plant (as early as 1884), causing cirrhosis of the liver and death to horses ingesting it (1, 2). Similar findings were reported by other authors (3). Of the more than 40 species of *Crotalaria* investigated to date, all were found to contain pyrrolizidine alkaloids (4). Many of these alkaloids cause hepatotoxicity as well as possessing antitumor properties (5). It appeared obvious that *C. sagittalis* would contain pyrrolizidine alkaloids as the toxic constituents. It was surprising to find that the first *Crotalaria* species implicated in crotalism was never investigated. This species is indigenous to a wide area of eastern North America and occurs only sporadically in Connecticut.

In the present investigation, the seeds were extracted with methanol and the total alkaloidal fraction was separated into free base and *N*-oxide fractions by partitioning between chloroform and water. The major alkaloid present in the chloroform fraction was isolated by acid extraction, basification to pH 9, and chloroform extraction. It was identified as monocrotaline by IR, NMR, and mass spectroscopy (MS). Reduction of the aqueous fraction with zinc dust also afforded monocrotaline, which was present in the plant as the major *N*-oxide. TLC analysis of the methanolic extract revealed the presence of three other pyrrolizidine alkaloids as well as the *N*-oxide of one of them. These are under investigation.

EXPERIMENTAL

Whole mature plants of *C. sagittalis* L.¹ were collected during the fall in Granby, Conn. The pods were removed, air dried, and coarsely ground in a ball mill. The material was extracted with petroleum ether (30-60°) to remove the waxy seed coat, and the dried marc was ground through a 40-mesh screen in a Wiley mill.

In a large conical percolator, 2.5 kg. of the defatted ground pods was continuously extracted with methanol until negative to

Dragendorff's reagent. The ethanolic extract was concentrated *in vacuo* to 2 l. One liter was concentrated to 500 ml. and an equal volume of water was added; extraction with petroleum ether (30-60°) was performed until the washings were colorless. TLC of the methanolic layer, using methods previously described (6), revealed four pyrrolizidine alkaloid free bases, *R_f*'s (9:1 MeOH-NH₃ on silica gel G) 0.27, 0.35, 0.45, and 0.56, and *N*-oxides, *R_f*'s 0.17 and 0.66.

The methanolic fraction was extracted with chloroform (4 × 100 ml.). TLC showed the presence of the free bases, which were extracted with 2 *N* H₂SO₄ until the washings were negative to Mayer's reagent. The acid fraction was adjusted to pH 5 with 28% NH₄OH, extracted with chloroform, adjusted to pH 9, extracted again, and finally adjusted to pH 12. Most of the alkaloid was present in the pH 9 chloroform extract, which on concentration afforded 625 mg. of solid. Two recrystallizations from acetone gave white crystals (m.p. 199-200°; yield 200 mg., 0.008% of the dried pods). A mixed melting point with authentic monocrotaline was not depressed. The IR (KBr), NMR (CDCl₃), and MS spectra were in agreement with those reported (7).

The aqueous methanolic fraction, previously extracted with chloroform and shown to contain the *N*-oxides by TLC, was adjusted to pH 2 with 2 *N* H₂SO₄ and stirred with an excess of zinc dust for 4 hr. The filtered solution was made basic (pH 9) with 28% NH₄OH and extracted with chloroform. Concentration gave 1.54 g. of tan solid, which was twice recrystallized from acetone to give monocrotaline (m.p. 199-201°; yield 754 mg., 0.06% of the dried pods) identical to the previous material. The other fractions are under investigation.

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▲ To whom inquiries should be directed.

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