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TROPANE ALKALOIDS AND TOXICITY OF *CONVOLVULUS ARVENSIS*

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Key Word Index—*Convolvulus arvensis*; Convolvulaceae; bindweed; tropane alkaloids; tropine; pseudotropine; tropinone; cuscohygrine; hygrine; 2-*C*-methylerythritol; toxicity.

Abstract—Horses in a few, localized northern Colorado pastures exhibited weight loss and colic. At post mortem, intestinal fibrosis and vascular sclerosis of the small intestine was identified. The pastures where the affected horses grazed were overrun by field bindweed (*Convolvulus arvensis*). Bindweed from the pasture was found to contain the tropane alkaloids tropine, pseudotropine, and tropinone and the pyrrolidine alkaloids cuscohygrine and hygrine. Laboratory mice readily ate *C. arvensis* and exhibited a variety of abnormal clinical signs depending on the amount eaten. Similar alkaloids have been found in other *Convolvulus* species and cuscohygrine and calystegines (polyhydroxytropanes) have been previously reported from *C. arvensis* roots. This is the first report of simple tropane alkaloids in *C. arvensis*, a world wide problem weed. Pseudotropine, the major alkaloid, is known to affect motility and might represent a causative agent for the observed cases of equine intestinal fibrosis.

INTRODUCTION

Thirteen unrelated horses with similar disease symptoms and originating from a localized area in northern Colorado were referred to the Colorado State University Veterinary Teaching Hospital from 1989 to 1994. All had a history of progressive weight loss and recurrent abdominal pain. Rectal palpation and laparotomy revealed the wall of the small intestine to be markedly thickened [1]. All horses were euthanized and post mortem examination showed marked small intestinal fibrosis. The geographic clustering of the cases (six different farms within a 30 mile radius) suggested an environmental causative factor in the disease. Inspection revealed poorly maintained pastures with little grass and common weeds. The primary source of water on two farms was surface water in ponds [1]. Water analysis of the ponds from the farms showed heavy metal concentrations to be in the safe range for livestock.

In the early spring of 1991, inspection of a farm from which five of the horses originated revealed that the main horse pasture was overrun by field bindweed, *Convolvulus arvensis* L. All affected horses at the other locations also had access to bindweed. Cuscohygrine [2] and calystegines [3] have been reported from roots of *C. arvensis*, but the only report found on the aerial parts [4] indicated the presence of alkanes, alkanols, α-amyrin, campesterol, stigmasterol, and sitosterol. Chemical and biological studies of above ground plant material of *C. arvensis* from one of the pastures where affected horses were kept were undertaken in order to determine causes of the animal toxicity.

RESULTS AND DISCUSSION

Fresh aerial parts (100 g) of *Convolvulus arvensis* yielded 17 mg of a crude base fraction which gave three alkaloid-positive bands on prep. TLC. The major alkaloid of the middle R_f band was shown to be pseudotropine (**1**) by GC R_t (7.9 min) and fragmentation pattern in comparison with a standard sample. A trace component of the band was tropine (**2**) identified by comparison of GC R_t (7.6 min) and fragmentation pattern with a commerical sample. Confirmation was achieved by reducing commercial tropinone (**3**), with $NaBH_4$ [5] to yield a 1:1 mixture of **1** and **2**, the GC–MS of which was compared to that of the isolate mixture. The major alkaloid of the lowest band was meso-cuscohygrine, (**4**) identified by R_t (11.1 min) and fragmentation pattern in comparison with a standard sample. This band also contained a trace amount of an alkaloid (R_t 11.4 min) with a mass spectrum very similar to that of **4** and probably was a cuscohygrine stereoisomer. The uppermost band had only a trace of alkaloid material, which was shown to be tropinone (R_t 7.4 min), identical with the commercial sample. Hygrine (**5**), (R_t 6.0 min), was also found to be present in trace amounts by GC–MS analysis of the total crude alkaloid extract and comparison with a standard.

Calystegines (such as calystegine B_2, **6**) cannot be identified by the above procedures so cation exchange column chromatography (Dowex 50WX8 resin, NH_4 form) [3, 6, 7] and preparative TLC was performed on a

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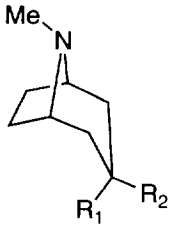

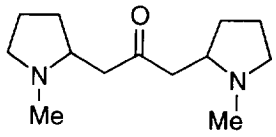

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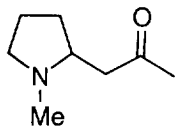

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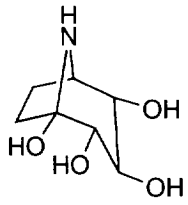
1: $R_1 = H$, $R_2 = OH$
2: $R_1 = OH$, $R_2 = H$
3: R_1, $R_2 = =O$
7: $R_1 = O_2CCH(CH_2OH)C_6H_5$, $R_2 = H$



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methanol Soxhlet extract of *C. arvensis*. The latter yielded 2-*C*-methylerythritol, first isolated from *C. glomeratus* [8]. No bioactivity data are known for this rare sugar, but its 2,4-cyclopyrophosphate derivative is accumulated in large amounts by bacteria undergoing oxidative stress [9]. Nothing which appeared to be a calystegine could be isolated by either method. Since these compounds are potent glycosidase inhibitors, bands scraped from preparative TLC of the methanol extract were tested for such activity, but proved negative or extremely weak (A. Elbein, private communication). Thus, we were unable to demonstrate the presence of calystegines in above-ground parts of *C. arvensis*. Pseudotropine (**1**) has been suggested to be the biosynthetic precursor of the calystegines [6, 10].

Mice fed exclusively bindweed ate the plant readily. In high dose trials, the mice showed no abnormalities until day 4, at which time four mice exhibited excitable behaviour, followed by depression, anorexia, hunched posture and death. By day 6 all had developed clinical signs and died or were killed. Complete post mortem examinations were performed. Gross findings were dehydration, retention of plant material in the stomach and gas distention of the small intestine. Histologic lesions were found in stomachs and livers. Of the 25 mice, eight had acute gastritis with multifocal erosions and ulcerations, eight mice had multiple large randomly located foci of acute hepatic necrosis. Two animals had both hepatic and gastric lesions. The other seven mice had no microscopic lesions. Mice fed bindweed and free choice standard diet showed no clinical abnormalities and were killed after 6 and 8 weeks. They had no gross lesions, but two had mild acute gastritis, six had mild multifocal hepatitis, and one had gastritis and hepatitis. No mice developed intestinal fibrosis.

Although the intestinal fibrosis in horses could not be reproduced in the mouse model, the clinical signs and morphologic lesions indicate that the mice had decreased motility in their gastrointestinal tracts. The hepatic lesions could be caused by absorption of toxins from the intestinal tract as a result of intestinal stasis.

Tropane alkaloids, such as atropine (**7**), are well known antimuscarinic agents. In particular, **7** depresses normal interdigestive secretion and all phases of the gastric response to food in the dog [11]. Atropine also inhibits transit through the small intestine in mice [12]. Pseudotropine (**1**) has been much less studied than **7**, but was found [13] to produce a sharp contraction of the intestine in the dog, followed by diminution of tone and inhibition of spontaneous contractions. Tropine (**2**) has a more or less opposite effect and augments contractions of intestinal muscles [13]. Cuscohygrine was found [14] to suppress an immune response in mice, but no other bioactivity reports on this alkaloid were found in the literature [15].

The total alkaloid content of *C. arvensis* was relatively low and acute poisoning has not been seen in horses, although this was the case with mice. Pseudotropine is not particularly toxic i.v. ($LD_{50} = 164\ mg\ kg^{-1}$ in mice [16]), but its oral toxicity is unknown. The disease syndrome in horses [1] appears to be the result of a chronic, low-level environmental insult rather than an acute poison. The pharmacological data discussed above suggest that the chronic ingestion of tropane alkaloids of bindweed may affect intestinal processes and could be the causative agents of the horse poisoning. Bindweed does not appear to be particularly palatable to animals, but horses will readily eat the plant if hungry. Its pervasive occurrence in pastures and as a worldwide contaminant in hay suggests that it could be an unrecognized, more wide-spread hazard beyond the localized case described here.

EXPERIMENTAL

Plant collection and isolation. Convolvulus arvensis was collected from a pasture on which the affected horses were kept in northern Colorado and its identity was confirmed by Professor D. Wilkin (Department of Biology, Colorado State University). The aerial parts (107 g wet wt) were extracted at room temp. for 24 hr with MeOH. The mixture was filtered and the MeOH evapd to leave about 25 ml of gummy solution which was taken up in 250 ml of

0.5 M aq. HCl. The solution was extracted with Et_2O (6 × 75 ml) and then with $CHCl_3$ (6 × 50 ml) to remove nonbasic material. The solution was made basic to pH 11 with K_2CO_3 and then extracted with $CHCl_3$ (6 × 50 ml). The dried (Na_2SO_4) $CHCl_3$ solution was evapd to yield 17 mg of crude alkaloid residue. The residue was examined by GC–MS and also as follows. The residue was dissolved in 1 ml of MeOH, streaked on a silica gel plate and the plate double developed ($CHCl_3$–MeOH, 4:1). The edge of the plate was sprayed with iodoplatinate to reveal the alkaloid-containing bands. Each band was scraped off and eluted with 1% aq. NH_3 in MeOH. The solutions were analysed by GC–MS: Hewlett-Packard 5890 GC (12 m × 0.2 mm i.d. HP1 column; 100% dimethylpolysiloxane; 2 min at 80°, then 10°min^{-1} to 140°, followed by 50°min^{-1} to 275° and hold for 3 min) connected to an HP 5970 quadrupole mass spectrometer (70 eV). Under these conditions, alkaloid retention times (min) were hygrine (6.0), tropinone (7.4), tropine (7.6), pseudotropine (7.9), cuscohygrine stereoisomer (11.1), cuscohygrine (11.4). Standard samples of pseudotropine, cuscohygrine and hygrine were from the laboratories of E. Leete (University of Minnesota). Tropine and tropinone were commercial samples and a mixture of tropine and pseudotropine was also obtained by reduction of tropinone [5].

A methanol Soxhlet extract of 76 g of dry *C. arvensis* yielded 5 g of gummy residue. Prep. TLC of a portion (silica gel, MeOH–$CHCl_3$–NH_4OH–H_2O, 46:50:1:3, double developed) and sprayed with ninhydrin showed a yellowish-brown band at R_f 0.69, a typical colour for calystegines [3]. Elution of this component and ^1H and ^{13}C NMR analysis of the residue showed it to be essentially pure 2-*C*-methylerythritol by comparison with literature data [8] as well as by mass spectra of the triacetate and tetra-*O*-trimethylsilyl ether derivative. Total yield was estimated at about 3 g of 2-*C*-methylerythritol.

Bioassays. Twenty 1-month-old male mice were fed fresh *C. arvensis* as their sole diet, while 10 mice were given a standard mouse diet. Since all mice eating bindweed developed toxic symptoms, the experiment was repeated with five mice and five controls. These mice also died or were killed by the sixth day of feeding and exhibited the symptoms described above. For lower dose studies, 10 mice were fed *C. arvensis* exclusively on Monday, Wednesday and Friday and standard diet the other days. Ten mice were also fed *C. arvensis* exclusively for two consecutive days, alternating with standard diet for two days, while six mice were offered bindweed free choice along with standard diet. No clinical abnormalities were observed during the 6–8 weeks of the feeding study. Gastritis and/or hepatitis was found in nine of the mice. In all the feeding trials, approximately 1.5 g of fresh plant material was provided to each mouse daily, but not all was consumed.

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