ISOLATION OF GALEGINE FROM *VERBESINA ENCELOIODES*

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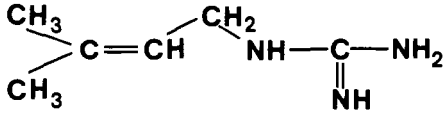
Verbesina enceloiodes (Benth. & Hook) (Compositae), a plant native to North America, is naturalized in southern and central Queensland, New South Wales, and Northern Victoria, where it is often found in great abundance. It is reported as causing serious sheep and cattle losses (1). Sheep are the animals most commonly affected, and field evidence indicates that most cases occur during drought or in animals introduced to new pastures. Most of the poisoned animals die suddenly, and post mortem examinations consistently show congestion of the lungs and a large amount of clear straw-colored fluid in the chest cavity. The plant is reported to contain potentially toxic levels of nitrate (2), but the syndrome is not consistent with nitrate or nitrite poisoning (1). The following article records the isolation of a toxic principle from *Verbesina enceloiodes* and its identification as galegine, 3-methyl-2-butenylguanidine (**1**).



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Toxicity testing was by ip injection of female mice (25–30 g) with sterile aqueous solutions of the toxin adjusted to pH 7. Two sheep used in a large animal toxicity test were dosed by stomach tube with dilute aqueous solutions of the toxin.

The dried powdered plant (200 g), Queensland Herbarium No. BRI 1087578, was extracted 3 times with hot methanol-water (1:1) and the extract concentrated under reduced pressure to a thick syrup. This was extracted twice with ether then four times with *n*-butanol. The *n*-butanol extract was concentrated to dryness under reduced pressure at 50°; the residue was dissolved in acetic acid-water (70:30, 10 ml) and Hyflo Supercel (Johns-Manville) (15 g) added. The mixture was applied to a column (4 x 50 cm) prepared by saturating Hyflo Supercel (30 g) with acetic acid-water (70:30) (24 ml) slurrying with toluene and packing under 10 lb/sq. in. pressure. The column was eluted with toluene (200 ml) followed by increasing quantities of chloroform in toluene (each 200 ml). In each case the eluting solvent was saturated with ⅓ of its volume of acetic acid-water (70:30). Fractions containing the toxin were combined, activated carbon (10 g) added, and the mixture stirred mechanically for 30 min. After filtering the carbon was washed with methanol, and the filtrate and washings were concentrated to dryness under reduced pressure. The toxin was then purified by silicic acid chromatography; the elution was started with 10% methanol in chloroform and followed by increasing quantities of methanol in chloroform. A white toxic solid (0.5 g) resulted, Rf 0.75 chloroform-methanol-acetic acid-water (65:25:5:5), and Rf 0.65 ethanol-1N ammonium hydroxide solution (80:20) on Silica gel G plates 0.25 mm thick. The toxin was revealed on the plates by exposure to gaseous Cl_2 followed by spraying with Starch-I_2.

The toxic compound was identified as galegine (**1**) by comparison of its

^{1}H nmr and mass spectra (m^+ *m/z* 127, $C_6H_{13}N_3$) and chromatographic properties with an authentic synthetic sample of galegine (4).

The symptoms and pathological lesions of sheep poisoned with *Verbesina enceloiodes* (1) closely resemble those reported for *Galega officinalis* (Leguminosae) (goats rue), a perennial herb growing in the Middle East and North America (3). Goats rue has been shown to contain 0.1–0.3% galegine and is responsible for stock losses in these areas. The isolation of galegine from *Verbesina enceloiodes* is the first report of this compound found in another family.

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