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# GALEGINE AND A NEW DIHYDROXYALKYLACETAMIDE FROM *VERBESINA ENCELOIODES*

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**Key Word Index**—*Verbesina enceloiodes*; Compositae; galegine; *N*-(2,3-dihydroxy-3-methylbutyl)-acetamide; synthesis; toxicity.

**Abstract**—The toxic principle from *Verbesina enceloiodes* has been identified as galegine (3-methyl-2-butenylguanidine). A novel co-occurring non-toxic extractive was shown to be *N*-(2,3-dihydroxy-3-methylbutyl)-acetamide. The structures assigned to both compounds were confirmed by synthesis.

## INTRODUCTION

*Verbesina enceloiodes*, 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 having been responsible for serious cattle and sheep losses in these areas [1]. Sheep are the most commonly affected animal and field evidence indicates that many cases occur during drought or amongst animals introduced to new pastures. Most of the affected animals die suddenly and post-mortem examinations consistently show congestion of the lungs and a large amount of clear straw-coloured fluid in the chest cavity. The plant has been reported to contain potentially toxic amounts of nitrate [2] but the syndrome is not consistent with nitrate or nitrite poisoning [1].

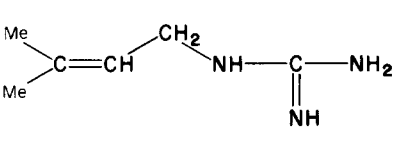
This paper reports the isolation of the toxic principle from *V. enceloiodes* and its identification as galegine **1** by comparison with a synthetic sample. Extracted together with galegine was a non-toxic hydroxylated *N*-alkylacetamide whose assigned structure **2** was also confirmed by synthesis.

## RESULTS AND DISCUSSION

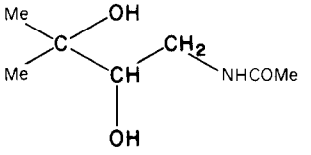
Extraction of the dried aerial parts of *V. enceloiodes* for the toxic constituent was monitored at all stages by toxicity testing against mice. The final purified fraction containing the toxin could be separated on a silicic acid column from a non-toxic compound which co-extracted with the toxin. The $^1$H NMR spectrum of the toxic fraction in $CD_3OD$ showed two olefinic Me signals at $\delta$ 1.71 and 1.76 together with one olefinic proton as a triplet of triplets at $\delta$ 5.26 coupled ($J$ = 7, 1.5 Hz) to an adjacent methylene group (*br d* at $\delta$ 3.78) indicative of a dimethylallyl moiety attached to an electronegative atom or group. The remaining large singlet at $\delta$ 4.78 in the spectrum was due to proton exchange of the compound with the solvent. The probe mass spectrum of the toxin showed the highest mass ion at $m/z$ 127 which measured for $C_6H_{13}N_3$. In the CIMS($NH_3$) spectrum this ion had shifted to $m/z$ 128 confirming that it was the $M^+$ of the compound. Combining both sets of spectroscopic data led to the conclusion that the compound must be 3-methyl-2-butenylguanidine **1**, commonly known as galegine [3].

The assignment of galegine was confirmed by synthesis using a published route [5] and comparison of the chromatographic and spectroscopic properties of the synthetic and naturally occurring compounds which proved to be indistinguishable. Additionally, the symptoms and pathological lesions of sheep poisoned with *V. enceloiodes* [1] closely resemble those reported for *Galega officinalis* (goats rue) [4]. The latter is a perennial legume growing in the Middle East and North America which has been shown to contain 0.1–0.3% galegine [3] and is responsible for stock losses in these areas.

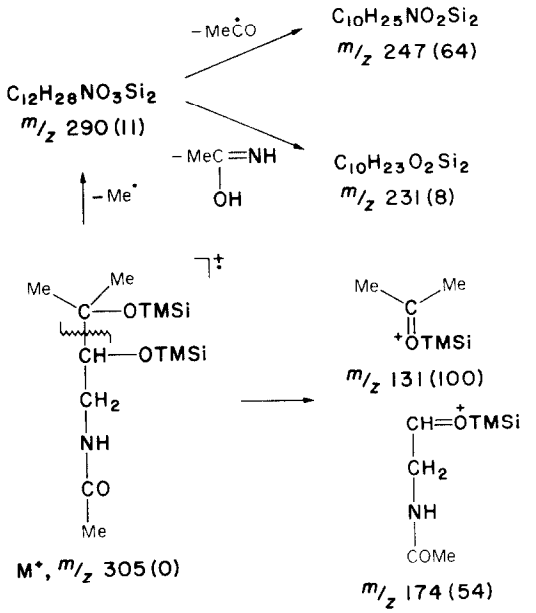
The compound which had similar chromatographic properties to galegine and co-extracted with it from *V. enceloiodes* was identified as *N*(2,3-dihydroxy-3-methylbutyl)-acetamide **2** by analysis of its $^1$H and $^{13}$C NMR spectra and the EIMS (70 eV) of its TMSi derivative. The latter showed an $[M—CH_3]^+$ ion for a di-TMSi derivative at $m/z$ 290 whose composition was confirmed as $C_{12}H_{28}NO_3Si_2$ by mass measurement, thereby establishing the composition of the compound itself as $C_7H_{15}NO_3$. The fragmentation pattern for the di-TMSi derivative was consistent with structure **2** (Scheme 1). Although the direct




**1**




**2**




Scheme 1.

probe EIMS of **2** showed no $M^+$ and only weak high mass ions for $[M—CH_3]^+$ and $[M—H_2O]^+$, at *m/z* 146 and 143, respectively, its CIMS($NH_3$) showed an abundant $[M + H]^+$ ion at *m/z* 162.

The $^1H$ NMR spectrum of this non-toxic compound, recorded in $CD_3OD$, showed a 6-proton singlet at $\delta$ 1.18, a $CH_3$ singlet at $\delta$ 1.96 and a multiplet between $\delta$ 2.7 and 3.5 which was partially obscured by the residual proton signals due to the methyl group of the solvent. In $d_6$-DMSO with added $D_2O$, the multiplet was revealed as a 3-proton ABC system ($\delta_A$ 3.43, $\delta_B$ 3.20, $\delta_C$ 2.79, $J_{AB} = +2.5$ Hz, $J_{AC} = -13.4$ Hz, $J_{BC} = +9.3$ Hz, $CH_BCH_AH_C$) while the 6-proton signal had split into two $CH_3$ singlets at $\delta$ 1.18 and 1.22. Also present in the $d_6$-DMSO spectrum were three broad one-proton signals at $\delta$ 4.36, 4.82 and 7.84 (2 × OH, NH) which disappeared after addition of $D_2O$. These assignments were in agreement with structure **2** for the non-toxic extractive, and were supported by its $^{13}C$ NMR spectrum in $CD_3OD$ which showed the two geminal $CH_3$ signals at $\delta$ 25.0, the acetyl $CH_3$ at $\delta$ 22.7 and the methylene, methine and quaternary C atoms at $\delta$ 41.9, 77.1 and 73.1, respectively. The carbonyl carbon was present at $\delta$ 174.9. The off-resonance $^{13}C$ NMR spectrum of the compound exhibited the appropriate signal multiplicities.

It remained only to confirm by synthesis the structure **2** assigned to the compound. For this purpose, 3-methyl-2-butenylamine hydrochloride, also used as an intermediate in the synthesis of galegine, was acetylated to give *N*-(3-methyl-2-butenyl)-acetamide. Hydroxylation of this compound using the Van Rheenan procedure [6] afforded racemic **2** in good yield. This was shown to be identical in all respects except optical rotation with the non-toxic compound isolated from *V. enceloiodes*. Work is under way to determine the absolute stereochemistry of the naturally-occurring compound.

There have been few reports of the isolation of simple amides from natural sources. *N*-3-Methylbutylacetamide has been identified as a volatile constituent in sherry [7], wine [8] and tobacco [9] and has also been characterized, together with a number of other aliphatic amides, as a component in the secretion from the rectal pheromone gland of the Queensland fruit fly [10]. We believe that this is the first reported instance of the occurrence of a hydroxylated alkylacetamide from a plant species although the isolation of 4-hydroxygalegine from the seeds of *G. officinalis* [11] has been previously reported.

## EXPERIMENTAL

$^1H$ NMR: 100 MHz, TMS as int. standard. $^{13}C$ NMR: 15.1 MHz, dioxane as int. standard. EIMS: 70 eV, direct probe. CIMS: 100 eV, direct probe, reagent gas as stated. Unless stated otherwise, Merck Si gel 60 (70–230 mesh) was used for CC.

Toxicity testing was by i.p. injection of female mice (25–30 g) with sterile aq. solns of the plant extracts adjusted to pH 7 and was used to monitor all stages of the isolation procedure. Two sheep used in a large animal toxicity test of the purified toxin were dosed by stomach tube with dilute aq. soln of the compound.

*Isolation of* **1** *and* **2** *from* V. enceloiodes. Aerial parts of the dried powdered plant (200 g), collected at Chinchilla in S.W. Queensland, Herbarium No. BRI 1087578, were extrd × 3 with hot MeOH–$H_2O$ (1:1) and the extract concd under red. pres. to a thick syrup. Then *n*-BuOH extract of this syrup was concd to dryness under red. pres. at 50°, the residue dissolved in HOAc–$H_2O$ 7:3, 10 ml) and Hyflo Supercel (Johns Manville) (15 g) added. The mixture was applied to a column (4 × 50 cm) prepared by saturating Hyflo Supercel (30 g) with HOAc–$H_2O$ (7:3) (24 ml), slurrying with toluene and packing under 1 kg/cm$^2$ pressure. The column was eluted with toluene (200 ml) followed by increasing quantities of $CHCl_3$ in toluene (each 200 ml). In every case the eluting solvent was equilibrated by shaking with 33% of its vol. of HOAc–$H_2O$ (7:3). Fractions containing the toxin were combined, activated C (10 g) added and the mixture stirred for 30 min. After filtering, the C was washed with MeOH, excess $NH_4OH$ added, and the soln concd to dryness under red. pres. The toxin was then purified by silicic acid chromatography starting the elution with 10% MeOH in $CHCl_3$ and using increasing quantities of MeOH in $CHCl_3$. Fractions (10 ml) were monitored by TLC using Si gel G plates and $CHCl_3$–MeOH–HOAc–$H_2O$ (13:5:1:1) as solvent. The toxin was collected in tubes 18–36. This was evapd to yield a white solid (0.5 g) $R_f$ 0.75 $CHCl_3$–MeOH–HOAc–$H_2O$ (13:5:1:1) and $R_f$ 0.65 EtOH–1 N $NH_4OH$ (4:1), identified as galegine **1**.

Tubes 10–17 when evapd gave a viscous residue $R_f$ 0.8 $CHCl_3$–MeOH–HOAc–$H_2O$ (13:5:1:1) which was shown to be the non-toxic compound **2**.

Both compounds were revealed on TLC plates by exposure to gaseous $Cl_2$ followed by spraying with starch $I_2$.

*Compound* **1**. $^1H$ NMR, see text. EIMS *m/z* (rel. int.): 127.1109 $[M]^+$ (62). Calc. for $C_6H_{13}N_3$: 127.1109, 126 $[M - H]^+$ (28), 112 $[M - CH_3]^+$ (100), 84 $[M - CH_3N_2]^+$ (90), 70 $[M - CH_3N_3]^+$ (68). CIMS($NH_3$) *m/z* (rel. int.): 128 $[M + H]^+$ (100).

*Compound* **2**. $[\alpha]_D^{25}$ −13.8° ($c = 0.98$, EtOH). $^1H$ and $^{13}C$ NMR, see text. EIMS *m/z* (rel. int.): 146 $[M - CH_3]^+$ (2), 143 $[M - H_2O]^+$ (3), 103 $[M - C_3H_6O]^+$ (34), 102 $[M - C_3H_7O]^+$ (43), 72 $[CH_2NHCOCH_3]^+$ (14). CIMS($NH_3$) *m/z* (rel. int.): 162 $[M + H]^+$ (100), 144 $[MH - H_2O]^+$ (25). The di-TMSi derivative of **2** was prepared using TRISIL. EIMS *m/z* (rel. int.): 290.1615 $[M - CH_3]^+$ (11). Calc. for $C_{12}H_{28}NO_3Si_2$: 290.1608. For diagnostic fragment ions, see Scheme 1. CIMS($CH_4$) *m/z* (rel. int.): 334 $[M + C_2H_5]^+$ (6), 306 $[M + H]^+$ (3), 216 $[M + H - TMSiOH]^+$ (100).

*Galegine* **1** was prepared from 3-methyl-2-butenylamine HCl according to ref. [5] and isolated as galegine sulphate mp 224°

uncorr. (lit. mp 223–225° [5]). The $^1$H NMR spectrum, EI and CIMS of synthetic galegine were identical to those given above for the toxic component from *V. enceloiodes*. On TLC in the solvent systems given above the synthetic and naturally occurring compounds were inseparable.

N-(3-*Methyl*-2-*butenyl*)*acetamide*. 3-Methyl-2-butenylamine HCl (1.1 g) was dissolved in an ice-cooled mixture of $Ac_2O$ (6 ml) and pyridine (5 ml) and left stirring overnight at room temp. The soln was then poured into ice–$H_2O$ (100 ml) and the aq. soln extrd with $CHCl_3$ (3 × 20 ml). The combined $CHCl_3$ extracts were washed successively with 1 M HCl, satd aq. $NaHCO_3$, $H_2O$, then dried ($MgSO_4$). After removal of solvent, the residue was purified by CC ($CHCl_3$–MeOH, 4:1), yielding *N*-(3-methyl-2-butenyl)-acetamide (930 mg) as an oil. Found: $M^+$, *m/z* 127.0997. Calc. for $C_7H_{13}NO$: 127.0997. $^1$H NMR ($CDCl_3$): $\delta$ 1.66 (3H, *s*, H-4), 1.70 (3H, *s*, H-4′), 1.96 (3H, *s*, $COCH_3$), 3.80 (2H, *t*, H-1), 5.19 (1H, *t*, $J$ = 7 Hz, H-2), 7.42 (1 H, *br s*, NH). On addition of $D_2O$, the signal at 7.42 slowly disappeared and the $CH_2$ triplet at $\delta$ 3.80 collapsed to a *d* ($J$ = 7 Hz). $^{13}$C NMR ($CDCl_3$, int. ref. dioxane) $\delta$ 170.8 (*s*, >CO), 136.1 (*s*, C-3), 120.8 (*d*, C-2), 38.0 (*t*, C-1), 25.9 (*q*, C-4), 23.3 (*q*, $COCH_3$), 18.1 (*q*, C-4′), EIMS *m/z* (rel. int.) 127 $[M]^+$ (52), 112 $[M - CH_3]^+$ (8), 84 $[M - CH_3CO]^+$ (32), 70 $[M - 57]^+$ (100).

N-(2,3-*Dihydroxy*-3-*methylbutyl*)*acetamide* **2**. *N*-(3-Methyl-2-butenyl)acetamide (0.5 g) was added to a cooled (5°) aq. soln (2 ml) of *N*-methylmorpholine-*N*-oxide–$2H_2O$ (730 mg) and $OsO_4$ (3 mg) and left to stir overnight at room temp. [6]. $H_2S$ gas was then bubbled through the soln giving a black ppt. After filtration, the filtrate was evapd to a small vol., $Me_2CO$ (50 ml) added, the soln dried ($Na_2SO_4$), filtered and the solvent removed under vacuum. The oily residue was purified by CC ($CHCl_3$–MeOH, 4:1) yielding **2** (580 mg) as a viscous oil. Found: $[M - CH_3]^+$, *m/z* 146.0816. Calc. for $C_6H_{12}NO_3$: 146.0817. The $^1$H and $^{13}$C NMR spectra of synthetic **2** were identical with those of the non-toxic component from *V. enceloiodes*, as also were its EIMS and that of its di-TMSi derivative.

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