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AN EFFICIENT METHOD FOR THE ISOLATION OF PERAMINE, AN INSECT FEEDING DETERRENT PRODUCED BY THE FUNGUS *ACREMONIUM LOLII*

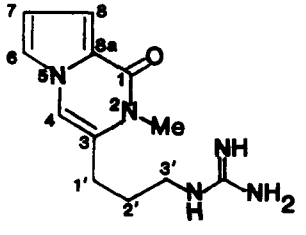
DARYL D. ROWAN* and BRIAN A. TAPPER

Biotechnology Division, Department of Scientific and Industrial Research, Private Bag, Palmerston North, New Zealand

Peramine [**1**], a novel fungal metabolite isolated from perennial ryegrass (*Lolium perenne* L.) infected with the fungal endophyte *Acremonium lolii* Latch, Christensen, Samuels (Fungi Imperfecti) (1,2), is a highly active feeding deterrent to the Argentine stem weevil (*Listronotus bonariensis* Kuschel) and is implicated in the resistance of endophyte-infected ryegrasses and fescues to this insect pest. The original isolation of peramine from herbage (1) required final purification as the diacetyl derivative (2) and was deemed impractical for producing sufficient underivatized material for further biological testing.

We now report an improved procedure for the isolation of peramine from seed which uses ion exchange and reversed-phase chromatography to take advantage of the strongly basic guanidino group and the lipophilic pyrrolopyrazine moieties present in peramine. This procedure has yielded for the first time pure crystalline peramine for which spectroscopic data is now reported. This data supports the 3-(3′-guanidinopropyl)-2-methylpyrrolo-[1,2-*a*]pyrazin-1(2H)-one structure originally proposed for peramine from the nmr analysis of the diacetyl derivative (2).

Tlc analysis of *A. lolii* infected seeds and seedlings indicated the presence of peramine in greater or comparable amounts to that found in herbage (1). The relative absence of pigments suggested seeds as a preferred source of peramine. In the new procedure, a crude EtOH extract from ryegrass seeds was defatted with petroleum ether and then passed sequentially through columns of a strong anion exchanger (Dowex 2 × 8), then a weak cation exchanger (Amberlite CG-50) linked in series. Peramine-containing fractions were eluted from the cation exchanger with HCOOH and concentrated before desalting and further purification on C-18 reversed-phase silica. In the presence of inorganic salts, peramine and associated pigments showed strong binding to the reversed-phase. Salts were washed from the C-18 column with H_2O and the peramine subsequently eluted by the addition of a small percentage of MeOH to the mobile phase. Peramine was precipitated as the picrate and subsequently recrystallized as either the sulfate or, preferably, the hydrobromide salt.



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Hrms of peramine HBr mp 242–243° confirmed $C_{12}H_{17}N_5O$ as the molecular formula of the free base. Fragmentation ions at *m/z* 85 (45%, $C_3H_7N_3$) and 73 (100%, $C_2H_7N_3$) as well as series of ions attributable to losses of NH_3, $NCNH_2$, $NH_2C(=NH)NH_2$, $CH_2NHC(=NH)$-NH_2, and $CH_2CH_2NHC(=NH)NH_2$ indicated an *n*-alkyl substituted guanidino group.

The ^1H nmr was analyzed using homodecoupling, COSY 45, and double quantum filtered COSY experiments and showed identical connectivities and similar coupling constants to those reported for diacetyl peramine (5). The lowest field methylene protons at δ 3.08

(2H, t, $J = 6.6$ Hz, H-3′) were assigned as adjacent to the guanidino group and were coupled to an adjacent methylene H-2′ (δ 1.73, quintet, $J = 7$ Hz) as suggested by hrms. The H-2′ protons showed further coupling to the H-1′ methylene protons at δ 2.51 (2H, t, $J = 7.4$ Hz) which in turn showed allylic long range coupling to an aromatic proton H-4 (δ 7.09). On resolution enhancement H-4 appeared as a quartet ($J = 0.8$ Hz, intensities 1:3:3:1) arising from the overlapping long range couplings, each of approximately 0.8 Hz, from both the H-1′ methylene protons and from a further aromatic proton H-8 (δ 6.82). H-8 was further coupled as part of a three-proton aromatic system (H-6, -7, -8) which showed chemical shifts (δ 7.13, 6.47, 6.82, respectively) and coupling constants ($J_{6,7} = 2.6$, $J_{6,8} = 1.5$, $J_{7,8} = 4.1$ Hz) typical of a 1,2-disubstituted pyrrole system (3). NOe difference experiments with diacetyl peramine (2) have previously established the relative positions of the aromatic and *N*-Me protons.

The ^{13}C nmr of peramine HBr showed the presence of one methyl, three methylene, four methine, and four quaternary carbons. The *N*-Me, carbonyl, and guanidino resonances (δ 30.3, 157.5, and 159.2, respectively) are consistent (4,5) with the proposed structure. DEPT and single frequency decoupling experiments were used to assign all protonated carbons while the quaternary carbons C-3 and C-8a were assigned by comparison with the synthetic model compound 2-methylpyrrolo[1,2-*a*]pyrazin-1(2H)-one (6). Some of the assignments previously reported (2) for diacetyl peramine should be revised.

Peramine deterred feeding by adult and larval Argentine stem weevil at concentrations down to 0.1 and 2 μg/g, respectively, and disrupted development of the cutworm *Graphania mutans* Walker at 10 μg/g (7). Further studies on the biological activity of peramine are continuing.

EXPERIMENTAL

^1H- and ^{13}C-nmr spectra were measured at 250 and 67.5 MHz, respectively, in D_2O using dioxane (δ_H 3.70, δ_c 67.4) as an internal standard.

EXTRACTION PROCEDURE.—High endophyte perennial ryegrass seed (Grasslands cultivar Ariki; voucher specimen CHR287857, Botany Division, DSIR, New Zealand) (20 kg) was coarsely milled and stirred for 24 h in 95% EtOH (100 liters). The EtOH extract was filtered off and the seed material re-extracted with a second batch of EtOH (80 liters). The combined EtOH extracts were filtered and concentrated to 15 liters in a climbing film evaporator. Five-liter portions of the concentrate were adjusted to a nominal 80% EtOH concentration with H_2O and partitioned with petroleum ether (2 liters). The EtOH phases were combined and concentrated to a volume of 7 liters. Batches (3.5 liters each) of this EtOH solution were passed sequentially through columns of Dowex 2 × 8 (OH^- form) (22 × 10 cm i.d. column) and Amberlite CG-50 (H^+ form) (30 cm × 7.8 cm i.d.) linked in series. The coupled ion exchange columns were washed with 50% MeOH until the effluent was almost colorless. The columns were separated and the peramine eluted from the Amberlite column with 5% HCOOH in 50% MeOH. Peramine-containing fractions, determined on tlc $CHCl_3$-MeOH-H_2O-HOAc (20:10:1:1) using Ehrlich's reagent (1), were combined and concentrated to 180 ml. The concentrate was added to a column of C-18 reversed-phase Si gel (Sepralyte™) (70 × 26 mm i.d.). The column was eluted with H_2O (30 ml) to remove salts and then with H_2O containing increasing proportions of 80% MeOH containing 5% HCOOH. Peramine eluted at between 6 and 12% MeOH. Addition of picric acid in EtOH to peramine-containing fractions precipitated the extremely fine picrate salt, which was centrifuged, filtered, and redissolved in warm EtOH for conversion to the sulfate using Dowex 2 × 8 (sulfate form). Peramine sulfate (20 mg, 0.001%) was recrystallized from H_2O and H_2O/EtOH as extremely fine white needles.

Peramine HBr, fine white needles, mp 242–243°, was prepared from the sulfate by ion exchange on Dowex 2 × 8 and recrystallization from 50% aqueous EtOH; uv λ max MeOH 231 (ε 33,100), 285 (8300) nm; ^1H nmr δ 1.73 (2H, quintet, $J = 7$ Hz, H-2′), 2.51 (2H, t, $J = 7.4$ Hz, H-1′), 3.08 (2H, t, $J = 6.6$ Hz, H-3′), 3.25 (3H, s, N-Me), 6.47 (1H, dd, $J = 2.5$, 4.1 Hz, H-7), 6.82 (1H, ddd, $J = 0.7$, 1.5, 4.1 Hz, H-

8), 7.09 (1H, br s, H-4), 7.13 (1H, dd, $J = 1.5$, 2.6 Hz, H-6); ^{13}C nmr δ 27.0 (C-2′), 27.8 (C-1′), 30.3 (N-Me), 40.9 (C-3′), 108.8 (C-4), 109.8 (C-8), 113.7 (C-7), 120.1 (C-6), 122.5 (C-8a), 128.1 (C-3), 157.5 (C-1), 159.2 (guanidino); eims *m/z* (rel. int.) $[M]^+$ 247.1433 (27) ($C_{12}H_{17}N_5O$ requires 247.1433), $[M-NH_3]^+$ 230.1199 (11), $[M-NCNH_2]^+$ 205.1233 (5), $[M-H_2NC(=NH)NH_2]^+$ 188.0939 (18), $[M-CH_2NH(C=NH)NH_2]^+$ 175.0863 (19), $[C_{10}H_9N_2O]$ 173.0722 (10), $[205-C_2H_4N]$ 163.0842 (10), $[205-C_2H_5N]$ 162.0792 (12), $[M-C_3H_8N_3]^+$ 161.0724 (12), $[C_7H_6N]$ 104.0509 (10), $[C_5H_4NO]$ 94.0289 (7), $[C_3H_8N_3]$ 86.0716 (33), $[C_3H_7N_3]$ 85.0641 (45), $[C_2H_7N_3]$ 73.0642 (100). *Anal.* calcd for $C_{12}H_{18}N_5OBr$: C 43.92, H 5.53, N 21.34; found C 43.48, H 6.21, N 21.03.

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