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A SALIVATION FACTOR FROM RHIZOCTONIA LEGUMINICOLA

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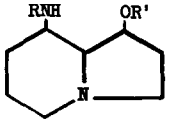
Isolation of a toxic alkaloid from Rhizoctonia leguminicola, a fungus infecting red clover forage, has been previously described (1,2). The present communication reports evidence supporting structure I (1-acetoxy-8-aminoindolizidine) for this compound.



I : R = H, R' = Ac

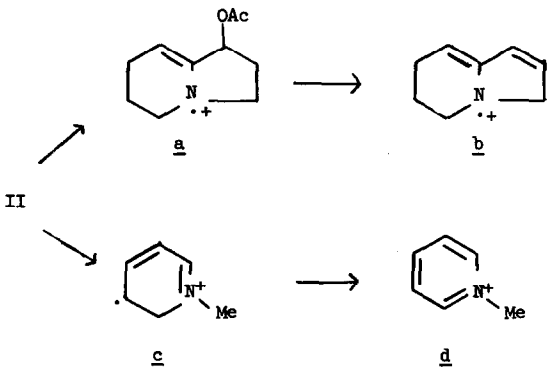
II : R = Ac, R' = Ac

III : R = Ac, R' = H

The alkaloid was purified as the dipicrate, $C_{10}H_{18}N_2O_2 \cdot 2C_6H_3N_3O_7 \cdot H_2O$ (Found: C, 39.29; H, 3.85; N, 16.18), m.p. 178-183°. The oily base, regenerated from the picrate, had $\lambda_{max}^{CCl_4}$ 5.77 and 8.1 μ (acetate ester). A weak parent ion in the mass spectrum at m/e 198 in addition to prominent P-60 and P-43 fragments (CH_3COOH and CH_3CO) confirmed the molecular formula of I.

With acetic anhydride I afforded an N-acetyl derivative (II), $C_{12}H_{20}N_2O_3$ (Found: C, 60.35; H, 8.55; N, 12.06), m.p. 143-146°, $\lambda_{max}^{CHCl_3}$

2.92, 6.06 and 6.68 (secondary amide), 5.82 and 8.15 (acetate ester) and 3.5 - 3.6 μ (Bohlmann bands (3)), but displaying no ultraviolet absorption, and inert to catalytic hydrogenation. Its mass spectrum showed a weak parent-ion peak at m/e 240 and stronger peaks at P-1 and P+1. A similar triplet pattern was observed for II-d_3 (prepared from I and acetic anhydride-d_6) at m/e 242-244, and for 1-ethyl-3-acetamidopiperidine at m/e 169-171. A prominent peak at m/e 181 (ion a), also found in the spectra of II-d_1 (prepared by mixing II with D_2O) and II-d_3, corresponded to loss of acetamide. Since no peak appeared at m/e 184 for II-d_3, O⟶N-acetyl migration does not occur in I, and the acetoxyl and amino groups are probably trans. The base peak at m/e 121 (ion b), unshifted for II-d_1 or II-d_3, arose from ion a by loss of acetic acid (metastable peak, m/e 81). An alternate mode of fission gave prominent peaks at m/e 95 (ion c) and 94 (ion d), related by a metastable peak at m/e 93.



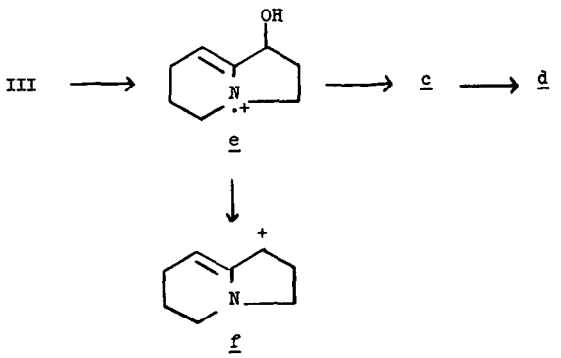
a b

c d

The n.m.r. spectrum of II had sharp singlets at τ 8.02 and 7.94 (CH_3CO), a multiplet centered at τ 7.0 corresponding to two equatorial protons (CHN<) (4), a broad doublet (J=8 cps) at τ 5.85 ($CHNHCOCH_3$), a complex multiplet at τ 4.80 ($CHOCOCH_3$), a broad doublet (J=8 cps) at

τ 3.69 (N<u>H</u>COCH$_3$). Upon addition of D_2O the last vanished and the doublet at τ 5.85 collapsed to an unresolved multiplet whose half-band width (10 cps) suggests that the proton C<u>H</u>NDCOCH$_3$, which is coupled with three other protons, is in an equatorial position on the 6-membered ring (5). Such conformational stability of the axial acetamido group implies that the adjacent C9-Cl bond is equatorial.

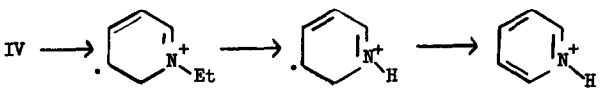
Treatment of II with potassium carbonate in methanol gave the alcohol (III), $C_{10}H_{18}N_2O_2$ (*Found:* C, 60.80; H, 9.10; N, 13.61), m.p. 157-158.5°, $\lambda_{max}^{CHCl_3}$ 2.92, 6.07, and 6.64 (secondary amide), 2.99 (hydroxyl), and 3.5 - 3.6 μ (Bohlmann bands (3)). Its mass spectrum showed a weak triplet in the parent-ion region (m/e 197-199), and the base peak at m/e 139 (ion <u>e</u>). The latter gave rise to ion <u>f</u> (low intensity peak at m/e 122; metastable peak at 107.3) or alternatively to ion <u>c</u> (m/e 95; metastable peak at m/e 65.2), thence to ion <u>d</u> (m/e 94; metastable peak at m/e 93.2). Loss of 44 m.u. to give an ion corresponding to ion <u>c</u> was observed as a primary fragmentation process of 1- and 2-hydroxyindolizidine.



The n.m.r. spectrum of III had a singlet at τ 8.08 (C<u>H</u>$_3$COO), six unassigned signals at τ>8.1 (C<u>H</u>$_2$ not adjacent to a heteroatom), two multiplets between τ 7.0 and 8.0 equivalent to five protons (C<u>H</u>N$\leq$) (4),

an unresolved multiplet at τ 5.92 (C<u>H</u>OH and C<u>H</u>NHCOCH$_3$), a broad singlet at τ 6.38 (O<u>H</u>), and a doublet (J=8 cps) at τ 2.8 (N<u>H</u>COCH$_3$). The latter two signals vanished upon addition of D_2O.

Alcohol III was oxidized with Jones' reagent (6) to ketone IV, $\lambda_{max}^{CHCl_3}$ 5.71 (5-membered ring ketone), 2.92, 3.01, and 6.62 (secondary amide), and 3.5 - 3.65 μ (Bohlmann bands (3)). The mass spectrum showed a moderately strong parent-ion peak at m/e 196. Loss of carbon monoxide and acetamide by both the alternate pathways (peaks at m/e 168 and 137; metastable peak at m/e 96) gave ion g (m/e 109; metastable peaks at 71 and 87) which in turn gave ion h (base peak at m/e 81; metastable peak at m/e 60.5), then ion i (m/e 80; metastable peak at m/e 79). Reaction of IV with D_2O for two days gave IV-d_3 (17%), IV-d_4 (5%), and negligible IV-d_5 (calculated (7) from peaks in the parent-ion and P-28 regions).



g h i

The ultraviolet spectrum (95% EtOH) of IV displayed only weak absorption in the 280-300 mμ region. Upon addition of alkali, intense maxima appeared immediately at 280 and 332 mμ owing to formation of the α,β-unsaturated 5-membered ring ketone by elimination of acetamide, possibly accompanied or followed by other changes. The products have not been isolated nor have the chromophores been otherwise identified.

Reaction of II with methyl iodide gave two stereoisomeric methiodides, V, m.p. 258-260°, and VI, m.p. 182-185° (major product), whose mass spectra correspond to superimposed spectra of II and methyl iodide. The n.m.r. spectrum of V in D_2O exhibited a sharp singlet at τ 6.70 (N-C<u>H</u>$_3$) in addition to peaks at τ7.83 and 8.03 (C<u>H</u>$_3$CO), the corresponding signals for VI

appearing at τ 6.85, 7.88 and 7.98, respectively. Indolizidine itself gives a 1:1 mixture of cis- and trans-methiodides with τ 6.88 and 7.12 (N-CH_3), respectively (8). Because of steric restrictions, tertiary azabicyclononanes other than indolizidine are not capable of forming two methiodides.

N.m.r. spectra (except of V and VI) were determined in $CDCl_3$ with an internal TMS reference on a Varian HA-100 spectrometer through the courtesy of Varian Associates and Dr. John Dickie of the Mellon Institute. Those of V and VI were determined in D_2O with an internal dioxane reference on a Varian A-60 spectrometer. Mass spectra were obtained on a CEC Model 21-103C spectrometer fitted with an all-glass inlet system heated to 200-250° (ionizing energy, 70 e.v.; current, 50 μa). We thank the Department of Chemistry for use of the latter two instruments, which were purchased from grants by the National Science Foundation, and Professor H. Whitlock for assistance in interpreting experimental results and obtaining mass spectra. Dr. H. S. Aaron, U. S. Army Edgewood Arsenal kindly provided samples of 1- and 2-hydroxyindolizidine. The work was supported in part by grants from the U. S. Public Health Service (No. AI-04419) and National Science Foundation (No. GB-4120).

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