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# (1S,2R,8R,8aR)-1,2,8-TRIHYDROXYOCTAHYDROINDOLIZINE (SWAINSONINE), AN α-MANNOSIDASE INHIBITOR FROM *RHIZOCTONIA LEGUMINICOLA*

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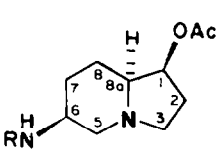
**Abstract**—The structure of an alkaloid isolated from *Rhizoctonia leguminicola*, which was previously assigned as 3,4,5-trihydroxyoctahydro-1-pyrindine, has now been revised to (1S,2R,8R,8aR)-1,2,8-trihydroxyoctahydroindolizine. The alkaloid is identical with swainsonine, obtained from the legume *Swainsona canescens*, and is a potent inhibitor of α-mannosidase.

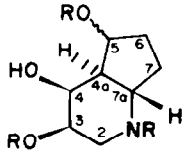
The fungus *Rhizoctonia leguminicola* has been a source of interest for a number of years due to its production of slaframine (**1a**), an alkaloid which causes excessive salivation in animals consuming mold-infested feeds. The structure, synthesis, biosynthesis and metabolism of this mycotoxin have been studied.[1]

In 1973, Guengerich *et al.* isolated a second alkaloid from the fungus and assigned its structure as 3,4,5-trihydroxyoctahydro-1-pyridine (**2**).[2] A triacetylated derivative was also prepared and given structure **3**.



**1a**, R = H

**b**, R = COCH$_3$



**2**, R = H

**3**, R = COCH$_3$

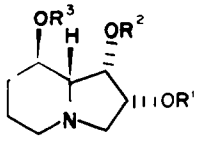
A further study of this new fungal metabolite has now been made. An improved isolation procedure enabled larger quantities of the alkaloid, as its triacetyl derivative, to be obtained from the mycelium. The alkaloid itself can be prepared from the triacetyl derivative by alcoholysis with $K_2CO_3$/MeOH followed by tlc purification.

A $^1$H-decoupled $^{13}$C NMR spectrum of the triacetyl derivative in $CDCl_3$ showed, as expected, 14 carbon atoms. Off-resonance $^{13}$C NMR studies, however, showed 4 doublets and 4 triplets in addition to signals for the three acetyl groups. This finding of 4 methine and 4 methylene carbons is incompatible with the previously assigned structures for the alkaloid (**2**) and its triacetyl derivative (**3**).

Study of the $^{13}$C chemical shifts and the $^1$H chemical shifts for the attached protons in the triacetyl derivative (deduced from a series of off-resonance spectra[3]) allowed assignments of the methine and methylene carbons. It was found that three of the methine carbons are bound to oxygen (δ68.01, 69.79, 70.17), while one is bound to nitrogen (δ69.20). Two of the methylene carbons are bound to nitrogen (δ51.70, 59.12), while the remaining two have only alkyl substituents (δ23.26, 29.76). The presence of three carbons bound to nitrogen indicates the alkaloid is a tertiary rather than a secondary amine.[2] Several additional observations indicate the presence of a tertiary amine in the triacetyl derivative: (i) the compound gives a positive reaction with Dragendorf reagent; (ii) the compound can be purified by extraction into aqueous acid and re-extraction into methylene chloride after basification; (iii) purified material fails to show an amide band in its infrared spectrum. Interestingly, the free alkaloid reacts with ninhydrin to give a purple color on heating, while the triacetyl derivative does not. This observation, in part, led to the original structure assignment as a secondary amine.[2] It should be noted, however, that other tertiary amines, such as 1-hydroxyoctahydroindolizine, also give a positive reaction with ninhydrin.

Examination of the $^1$H NMR spectrum of the triacetyl derivative (Fig. 1) aided by homonuclear decoupling gives rise to the partial structure shown in Fig. 2.

These chemical and spectroscopic findings in conjunction with the original data[2] lead to reassignment of the structure of the triacetyl derivative as 1,2,8-triacetoxyoctahydroindolizine (**4b**) and the alkaloid itself as the trihydroxy analog (**4a**).



**4a**, R$^1$ = R$^2$ = R$^3$ = H

**b**, R$^1$ = R$^2$ = R$^3$ = COCH$_3$

**c**, R$^1$, R$^2$ =  , R$^3$ = H

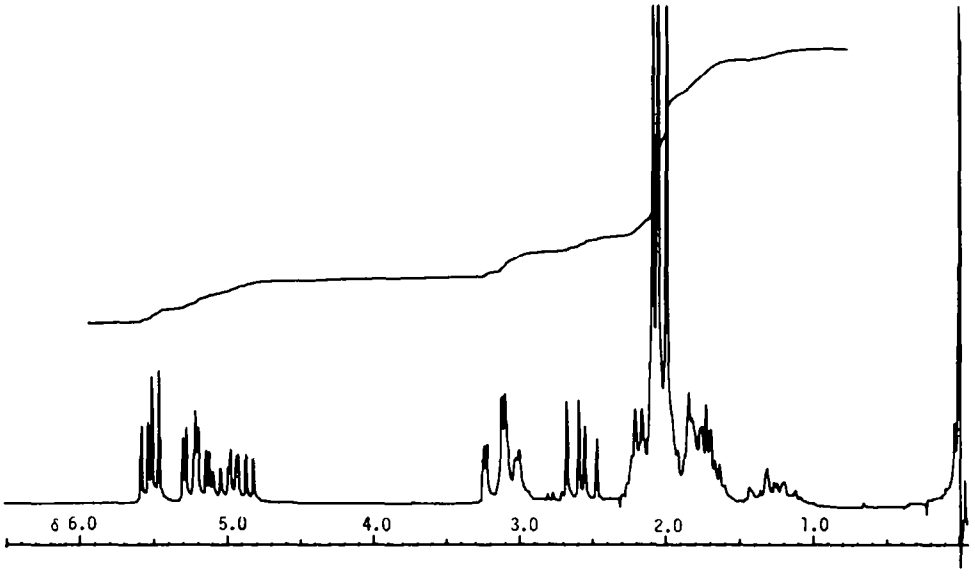



Fig. 1. $^1$H NMR of triacetyl derivative ($CDCl_3$).

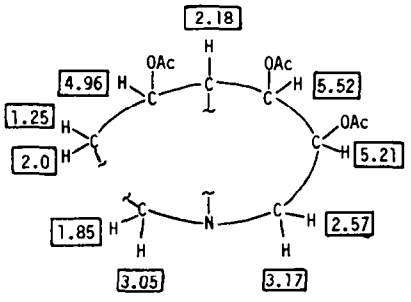
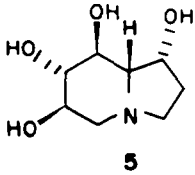



Fig. 2. Partial structure of triacetyl derivative.

The relative stereochemistry shown in structure **4** was established in the following manner. A *trans* ring junction was indicated by the presence of strong Bohlmann bands (2800 $cm^{-1}$) in the IR spectrum of the triacetate **4b**.[4] The *trans* diaxial relationship between H-8 and H-8a was clear from the large vicinal coupling constant ($J_{8,8a}$ = 9.6 Hz) in the $^1$H NMR spectrum of **4b**. The *cis* relationship between H-8a and H-1 was deduced by comparison of the vicinal coupling constant (**4b** $J_{8a,1}$ = 4.3 Hz) with those of model compounds, such as castanospermine **5** ($J_{8a,1}$ = 4.4 Hz), an alkaloid recently isolated from the seeds of *Castanospermum australe*.[5] The *cis* relationship of H-1 and H-2 in **4a** was demonstrated by formation of acetonide **4c** on heating **4a** with 2,2-dimethoxypropane and toluene-p-sulfonic acid.




**5**

The absolute configuration of **4a** was established by asymmetric induction using the method of Horeau.[6] Treatment of acetonide **4c** with racemic 2-phenylbutanoic anhydride yielded 2-phenylbutanoic acid as a byproduct of the acylation reaction. The acid had an enantiomeric excess of the dextrorotatory form indicating that C-8 has the R configuration. Therefore the complete structure of **4a** is (1S, 2R, 8R, 8aR)-1,2,8-trihydroxyoctahydroindolizine; the absolute configuration is as depicted in structure **4a**. The structural similarities of **4a** and slaframine (**1a**) are apparent; however, it should be noted that the two alkaloids are mirror images at C-8a and C-1. The biosynthetic origins of this structural difference will be the subject of a future communication.

After having arrived at structure **4a** for the alkaloid, a literature search brought forth the fact that swainsonine, an alkaloid having the same structure and relative configuration as **4a**, had recently been isolated from the Australian legume *Swainsona canescens*.[7] The spectra of the two alkaloids (IR, $^1$H and $^{13}$C NMR, MS) and of their triacetates (IR, $^1$H NMR, MS) were indistinguishable. The absolute configuration of swainsonine had not been established;[8] however, a comparison of optical rotations of the two alkaloids shows that the compounds are, in fact, identical rather than enantiomeric: **4a** $[\alpha]_D^{25} = -87.2°$ (*c* 2.1, MeOH), swainsonine[9] $[\alpha]_D^{20} = -78.9°$ (*c* 1.14, MeOH).

Swainsonine is a potent inhibitor of α-mannosidases and is the agent responsible for *Swainsona* toxicosis in livestock, a lysosomal storage disease involving accumulation of mannose-rich oligosaccharides.[10] The toxicosis mimics the genetic disease mannosidosis, a neurological condition in cattle and humans resulting from a severe deficiency of α-mannosidase.[11] Inhibition of α-mannosidase has been confirmed (Fig. 3) using the *Rhizoctonia* alkaloid. The hydrolysis of 4-methylumbelliferyl-α-D-mannopyranoside by jack bean α-mannosidase was 50% inhibited by 1.75 μM **4a**.

Dorling and coworkers have speculated that the inhibitory action of swainsonine results from structural similarity of its protonated form to the mannosyl cation, a proposed intermediate in enzymic mannosyl transfer reactions.[10] The absolute configuration, which has now been determined, supports their hypothesis; as shown in Fig. 4, C-2, C-1, and C-8a of protonated swainsonine are equivalent to C-2, C-3 and C-5, respectively, of the mannosyl cation. In this regard, swainsonine would ap-

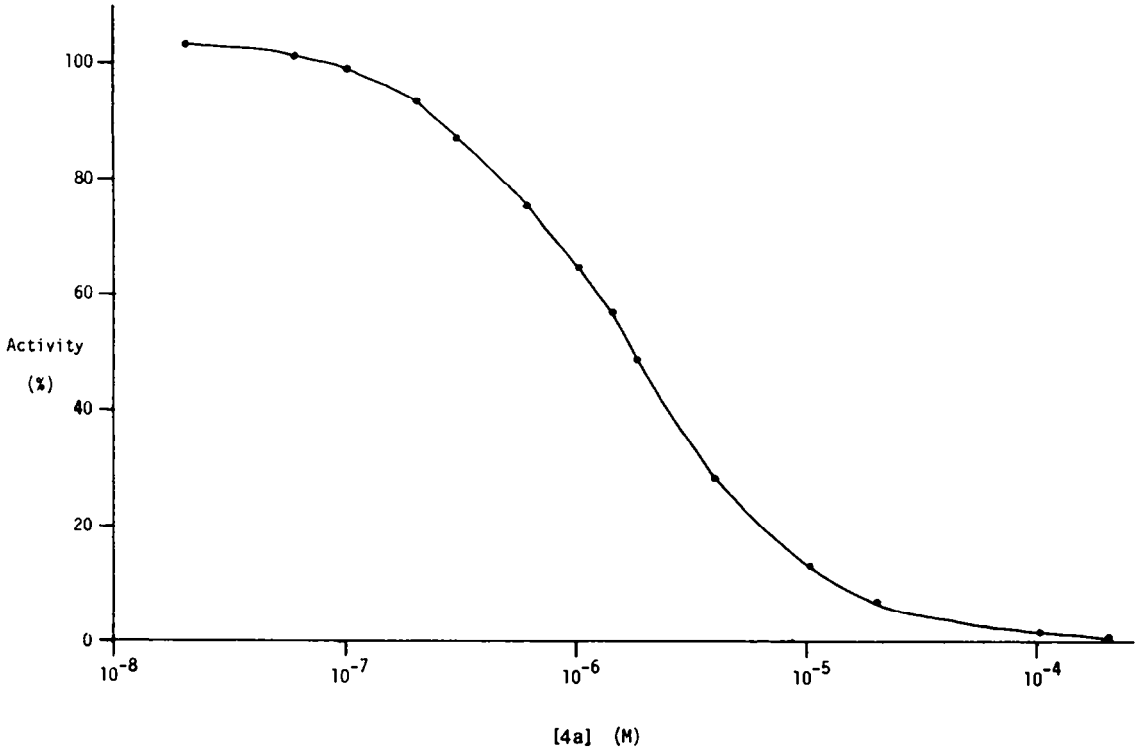



Fig. 3. Inhibition of jack bean α-mannosidase by **4a**. Assay is as described by Dorling *et al.*[10] Activity represents fluorescence relative to an uninhibited sample.

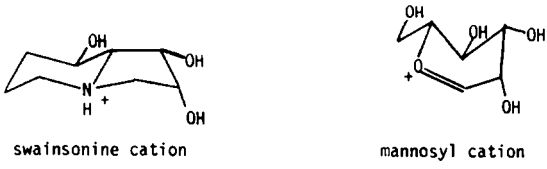
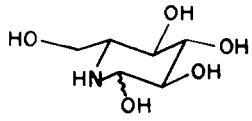



Fig. 4. Comparison of swainsonine and mannosyl cations.

pear to be similar to nojirimycin **6**, the 5-amino analog of glucose, which inhibits enzymic glucosyl transfer reactions.[12] A noteworthy difference between the two inhibitors is that nojirimycin is fully analogous to glucose, having equivalent chiral centers at C-2, C-3, C-4 and C-5, while swainsonine lacks the C-4 center of mannose.



**6**

## EXPERIMENTAL

Melting points were obtained with a Kofler hot stage and are uncorrected. Optical rotations were determined using a Rudolph Autopol III polarimeter, $^1H$ and $^{13}C$ NMR spectra were obtained with a JEOL FX-90Q NMR spectrometer, operating at 89.55 and 22.50 MHz, respectively. Infrared spectra were recorded on a Perkin-Elmer 621 IR spectrophotometer. Mass spectra were obtained with an LKB 9000 mass spectrometer (EI) and high resolution mass spectra were obtained with a VG Micromass 7070 mass spectrometer (CI, $NH_3$). Fluorescence was measured with an Aminco-Bowman spectrofluorimeter. Thin layer chromatography was carried out using Merck silica gel 60F-254 precoated glass plates, 25μ thickness. Silica gel for column chromatography was obtained from Davison (grade 62, 60-200 mesh). Short column chromatography[13] was carried out using Merck silica gel 60G (tlc grade).

*Maintenance of cultures*

*Rhizoctonia leguminicola* Gough *et* E. S. Elliott (ATCC 26280) was maintained on slants of 10% (wt/v) filtered red clover hay infusion containing 1.5% agar. A mycelial suspension (10 mL) from a slant served as inoculum for liquid cultures, grown in 1 liter Roux bottles containing the sterile red clover hay infusion (240 mL). These liquid cultures were incubated at 22–25° and the resultant mats subcultured every 3–4 weeks. A fresh culture line from a slant was begun every 3–4 months.

*Isolation of N-acetylslaframine* (**1b**) *and swainsonine triacetate* (**4b**)

Five 3-week old mycelial mats (wet wt = 47.5 g) were ground with 95% EtOH in a blender and extracted with EtOH (500 mL) in a Soxhlet apparatus for 24 h. The EtOH extract was concentrated under reduced pressure and the residue taken up in several small portions of water and filtered through glass wool. The aqueous solution, after extraction with $CH_2Cl_2$, was taken to pH 10 with $K_2CO_3$ and lyophilized. The residue was acetylated (50 mL $Ac_2O$, 48 h, 25°); the solvent was removed *in vacuo*. After addition of water and basification to pH 10 with $K_2CO_3$, the solution was extracted with $CH_2Cl_2$ which in turn was extracted with 1N HCl. The aqueous extract was basified with $K_2CO_3$ to pH 10 and extracted with $CH_2Cl_2$. Repetition of this extraction sequence eliminated the small remaining amounts of neutral contaminants. The final $CH_2Cl_2$ extract was dried ($Na_2SO_4$), filtered and evaporated to give the crude product mixture (39 mg). Compounds **1b** and **4b** were separated by short column chromatography (4% MeOH in $CHCl_3$) on silica gel. Each was then purified by a second short column (**1b**: 4% MeOH in $CHCl_3$, **4b**: 2% MeOH in $CHCl_3$). This procedure gave 8.1 mg of white solid **1b**; and 17.3 mg of **4b** as a pale yellow oil, $[\alpha]_D^{26} = +7.0°$ (*c* 1.77, MeOH); $^1H$ NMR ($CDCl_3$, ref: TMS) δ 5.52 (1H, dd, H-1, $J_{1,2} = 6.5$ Hz, $J_{1,8a} = 4.3$ Hz), 5.21 (1H, ddd, H-2, $J_{2,1} = 6.5$ Hz,

$J_{2,3'}$ = 7.5 Hz, $J_{2,3}$ = 2.0 Hz), 4.96 (1H, ddd, H-8, $J_{8,8a}$ = 9.6 Hz, $J_{8,7ax}$ = 10.7 Hz, $J_{8,7eq}$ = 4.75 Hz), 3.17 (1H, dd, H-3, $J_{3,2}$ = 2.0 Hz), 3.05 (1H, dd, H-5eq), 2.57 (1H, dd, H-3', $J_{3',2}$ = 7.5 Hz), 2.18 (1H, dd, H-8a, $J_{8a,1}$ = 4.3 Hz, $J_{8a,8}$ = 9.6 Hz), 2.08 (3H, s, $CH_3COO$), 2.05 (3H, s, $CH_3COO$), 2.0 (1H, m, H-7eq, $J_{7eq,8}$ = 4.75 Hz), 1.99 (3H, s, $CH_3COO$), 1.85 (1H, m, H-5ax), ~1.7 (2H, m, H-6ax, 6eq), 1.25 (1H, m, H-7ax, $J_{7ax,8}$ = 10.7 Hz); $^{13}C$ NMR ($CDCl_3$) $\delta$ 20.39 (q), 20.50(q), 20.88(q), 23.26(t), 29.76(t), 51.70(t), 59.12(t), 68.01(d), 69.20(d), 69.79(d), 70.17(d), 169.80(s), 170.02(s); IR(neat) 2800 (Bohlmann bands), 1750 (OAc), 1375, 1240 (C–O), 1040 $cm^{-1}$; MS *m/e* 239 (M-HOAc), 180 (M-HOAc, -OAc), 137 (M-2HOAc, $-CH_2{=}C{=}O$), 120 (M-2HOAc, -OAc); high resolution MS: found *m/e* 300.1428(M + H), calculated for $[C_{14}H_{22}NO_6]^+$ = 300.1447. In addition, 7.7 mg of a mixture of other compounds (largely corresponding to partially acetylated **4a**) was obtained, which could be acetylated using acetic anhydride and pyridine to give additional **4b**.

*Preparation of swainsonine* (**4a**)

(a) Compound **4b** (26 mg) and $K_2CO_3$ (20 mg) were stirred for 1.5 h in MeOH (4 mL) at room temperature. After concentration under reduced pressure, the mixture was spotted on two 20 × 20 cm analytical tlc plates and developed in acetone:$CHCl_3$:$H_2O$:conc $NH_4OH$ (75:12.5:10:2.5). The **4a** band on each plate (edge visualized with ninhydrin) was collected and eluted with MeOH. The eluate was concentrated to a small volume under reduced pressure and passed through a Millipore filter (type FH) to remove residual silica. The filtrate was evaporated under reduced pressure to give 10.4 mg (69% yield) of a yellow-white solid. Further purification could be obtained by sublimation *in vacuo* (80–100°C) to give **4a** as small white needles, mp 144–145°C (lit' mp 144–145°C); $[\alpha]_D^{25}$ = −87.2° (c 2.1, MeOH); $^1H$ NMR ($D_2O$, ref: DSS) $\delta$ 4.18–4.44 (2H, m), 3.80 (1H, ddd), 2.89 (2H, dd), 2.53 (1H, dd), 0.98–2.14 (6H, m); $^{13}C$ NMR ($D_2O$, ref: MeOH, 49.00) $\delta$ 22.89, 32.21, 51.38, 60.38, 66.06, 68.77, 69.42, 72.56; IR (KBr) 3200–3500 (OH), 2780–2820 (Bohlmann bands), 1060 $cm^{-1}$, MS *m/e* 173(M), 155, 138, 120, 113, 96.

(b) Compound **4a** could be obtained directly from the EtOH extract of the mycelium by thin layer chromatography (Rf ~ 0.3) as described in (a), although this method was less satisfactory for large scale preparations.

*Preparation of swainsonine acetonide* (**4c**)

A mixture of **4a** (10 mg), 2,2-dimethoxypropane (5 mL), and toluene-p-sulfonic acid (~40 mg) was refluxed for 1.25 h. The mixture was cooled; $CH_2Cl_2$ and $K_2CO_3$ were added. After filtration and evaporation under reduced pressure, the residue was purified using column chromatography with gradient elution (0–20% MeOH in $CHCl_3$). The product fractions were combined and evaporated under reduced pressure to give 8.6 mg of **4c** as a yellow-white solid (70% yield). $[\alpha]_D^{24}$ = −75.1° (c 1.54, MeOH); $^1H$ NMR ($CDCl_3$, ref: TMS) $\delta$ 4.54–4.78 (2H, m, H-1,2, $J_{1,2}$ = 6.3Hz, $J_{1\,8a}$ = 4.1 Hz, $J_{2,3'}$ = 4.0 Hz), 3.68–3.98 (1H, m, H-8), 3.15 (1H, d, H-3, J = 10.7 Hz), 2.98 (1H, br d, H-5eq), 2.13 (1H, m, 3', $J_{3',2}$ = 4.0 Hz), 1.9 (1H, m, 5ax), ~1.65 (1H, m, H-8a, $J_{8a,1}$ = 4.1 Hz), 1.51 (3H, s, $CH_3$), 1.34 (3H, s, $CH_3$), 1.1–2.2 (5H, m, H-6ax, 6eq, 7ax, 7eq, 8-OH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 24.04(t), 24.91(q), 26.00(q), 32.98(t), 51.59(t), 59.96(t), 67.44(d), 73.70(d), 78.30(d), 79.25(d), 111.45(s); IR (KBr) 3100–3500 (OH), 2780 (Bohlmann bands), 1055 $cm^{-1}$; MS *m/e* 213(M), 198, 156, 138, 126, 120, 113; high resolution MS found *m/e* 214.1431 (M + H), calculated for $[C_{11}H_{20}NO_3]^+$ = 214.1443. Sublimation gave colorless prisms, mp 105–107°C.

*Inhibition of jack bean α-mannosidase*

The general procedure of Dorling *et al.*[10] was followed. 100 μl of a 1:20,000 dilution (est. $4.5 \times 10^{-5}$ units)[14] of jack bean α-mannosidase (Sigma) was added to a mixture of 100 μl 15 *mM* 4-methylumbelliferyl α-D-mannopyranoside (Koch-Light) and 100 μl of various concentrations of **4a** in glass distilled water.

*Determination of absolute configuration of swainsonine acetonide* (**4c**)

A solution of 9.78 mg (0.046 mmol) of acetonide **4c** and 35 mg (0.11 mmol) of 2-phenylbutanoic anhydride in dry pyridine (0.2 ml) was allowed to stand at room temperature for 27 h. After addition of water and benzene, the mixture was titrated with 0.1 N NaOH to the phenolphthalein endpoint. The aqueous layer was acidified with conc HCl to pH 1 and extracted with benzene. The benzene was dried ($Na_2SO_4$) and evaporated under reduced pressure to give 27.3 mg of 2-phenylbutanoic acid. Benzene (1 ml) was added and a rotation of +0.434° was obtained in a 1 dm cell.

*Note added in proof.* After submission of this manuscript Molyneux Ind James reported[15] that swainsonine is also a metabolite of spotted locoweed (*Astragalus lentiginosus*), a toxic legume found in the western United States. Swainsonine strongly inhibits rat Golgi II and epididymal α-mannosidases as well as the lysosomal enzyme; it does not, however, inhibit the cytosolic enzyme nor the Golgi enzymes IA and B.[16]

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