(1S,2R,8aS)-1,2-Dihydroxyindolizidine Formation by *Rhizoctonia leguminicola*, the Fungus That Produces Slaframine and Swainsonine

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The title diol (**6a**) has been shown to be a metabolite of *Rhizoctonia leguminicola*, the fungus that produces two toxic indolizidine alkaloids, swainsonine (1) and slaframine (2). The structure of **6a** was established spectroscopically and confirmed by synthesis of both C-8a epimers **6a** and **7a**. The absolute configuration was inferred from the efficient reutilization of the isolated **6a** to form 1. Diol **7a** could not be detected among the products of fermentation. It is noteworthy that **6a** is probably an intermediate in the biosynthesis of swainsonine although it differs from the alkaloid in being epimeric at the 8a position.

The indolizidine alkaloid swainsonine[1] (1) has been shown to be the causative factor in locoism induced by the American locoweed[2,3] (*Astragalus lentiginosus* and related species) and the Australian Darling pea[4] (*Swainsona canescens*). The alkaloid has also been found as a metabolite of two microorganisms, *Rhizoctonia leguminicola*[5] and *Metarhizum anisopliae*.[6] In the fungus *R. leguminicola*, swainsonine occurs along with another indolizidine alkaloid, slaframine (2), which causes slobber syndrome in animals that ingest mold-infested feeds.[7]

The biosynthesis of swainsonine and slaframine by *R. leguminicola* presents an interesting problem because these two superficially similar alkaloids have opposing configurations at both C-1 and C-8a (Scheme I). Both alkaloids have been shown to arise from L-lysine, which cyclizes to form pipecolic acid (3).[8] All six of the pipecolate carbon atoms are incorporated into the final structure with the remaining two carbon atoms in the indolizidine nucleus, i.e., C-2 and C-3, being provided by malonate, presumably via a Claisen-type condensation to form pipecolyl acetate.[9] Cyclization and reduction give 1-oxoindolizidine (4), which is reduced to 1-hydroxyindolizidine (5). The cis form, 1-hydroxyindolizidine (**5a**), is involved in the formation of slaframine; **5a** is functionalized at C-6 and finally acetylated to give **2**.[7] A fundamental difference in the biosynthesis of swainsonine was recognized when a biosynthetic experiment carried out with DL-perdeuteriopipecolic acid led to swainsonine lacking deuterium at C-8a, whereas slaframine retained the isotopic label at that position.[10] Whether swainsonine also arises from **5a** or from the trans isomer (**5b**) has not yet been established. Moreover, the order of hydroxylation is unknown.

Incorporation of isotopic label from a mixture of *cis*- and *trans*-DL-[1-^{3}H]-1-hydroxyindolizidines (**5a,b**) into swainsonine as well as into slaframine has been demonstrated.[11] During fermentations using DL-[1-^{3}H]-**5a,b**, which were carried out to produce [^{3}H]swainsonine, minor tritiated metabolites were investigated because of the possibility that other indolizidines could be identified, in particular, compounds that might shed light on unknown steps in the swainsonine pathway. Chromatographic separations by ion-exchange revealed a minor metabolite, formed in less than 5% the yield of swainsonine. Mass spectra showed the compound to be a dihydroxyindolizidine and suggested that the hydroxy groups were vicinal at carbons 1 and 2, because the parent ion (m/z 157) fragmented to an ion at m/z 97; this fragmentation parallels that of swainsonine where the parent ion (m/z 173) yields a fragment at m/z 113.[1] Proposed assignments for these ions involving loss of C-1 and C-2 are shown in Scheme II. The diol is assigned as 1,2-cis (i.e., **6a** or **7a**) by its reduced mobility on a borate-impregnated TLC plate due to formation of a cyclic borate ester; swainsonine is also retarded by borate impregnation.

The diacetate (m/z 241) was prepared by treatment of the diol with acetic anhydride; exact mass measurement confirmed the empirical formula as $C_{12}H_{19}NO_4$. The diacetate is preferable to the diol for analysis of the NMR spectrum because it is easier to obtain in pure form and because acetylation of the alcohols deshields the α protons (H-1 and H-2) to give better spectral dispersion. The key assignments involve these two protons, which appear at δ 4.77 and 5.24, respectively. One of the H-3 protons is at 3.60, H-5$_{eq}$ is at 3.03, and the remaining protons lie between 1.0 and 2.4. The vicinal relationships of H-1 with H-2 and of H-2 with H-3 have been demonstrated by spin decoupling; irradiation of the signal at 5.24 collapses the multiplet at 4.77 to a doublet ($J_{1,8a}$ = 9 Hz), the one at 3.60 to a doublet ($J_{3,3'}$ = 10 Hz), and causes a visible change in the unresolved signals at 2.3 (H-3'). Irradiation at 4.77

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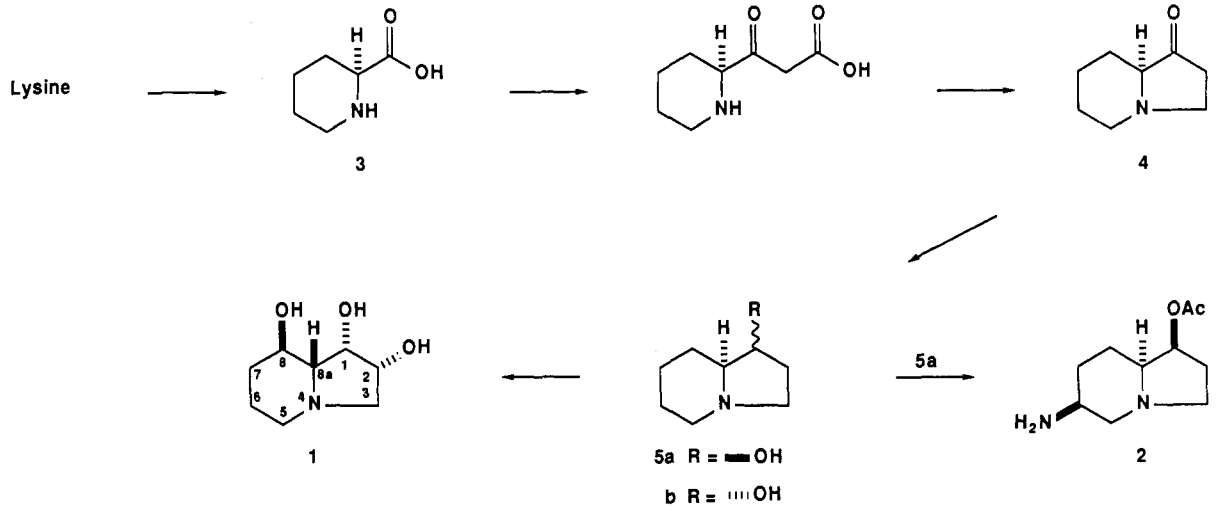
0022-3263/87/1952-3094$01.50/0

Scheme I



Scheme II

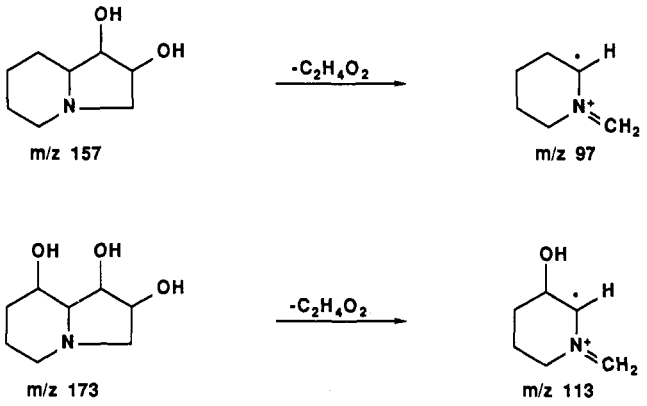
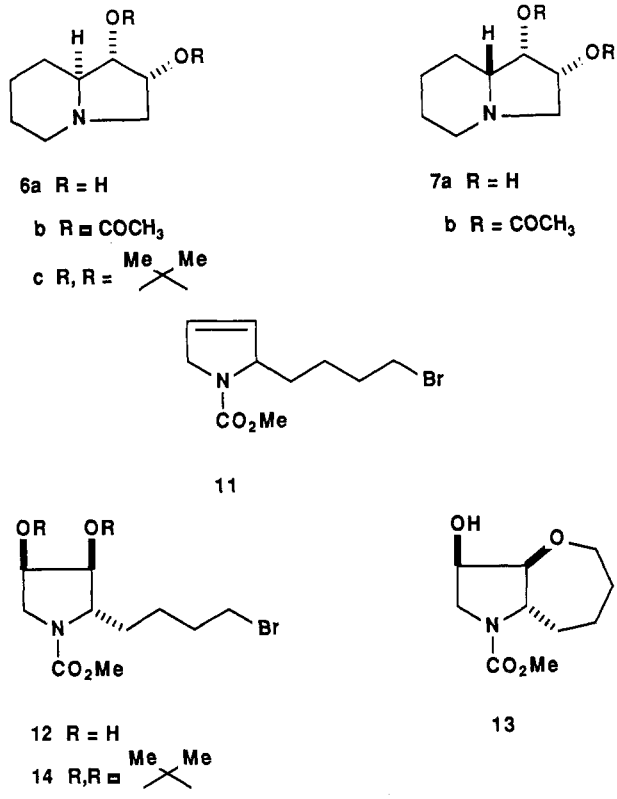


Chart I



collapses the signal at 5.24 into an unresolved hump. Irradiation at 3.60 collapses the signal at 5.24 into a broad hump and causes a visible change near 2.3.

The 9-Hz coupling constant between H-1 and H-8a points to a trans relationship (i.e., **6b** not **7b**). By comparison, the corresponding protons in swainsonine are cis and are coupled by 4.3 Hz.[5] However, stereochemical assignments based on vicinal coupling constants are insecure in five-membered rings because of uncertainty concerning conformations. Moreover, biosynthetic considerations made it more likely that the metabolite was **7a**. As a consequence, an authentic sample of **7a** was synthesized.

The synthesis of diol **7a** and the diacetate (**7b**) was based on swainsonine itself. The structure of swainsonine is completely secure; connectivities and relative configurations, originally assigned by chemical and spectroscopic means,[1,5] have been confirmed by single-crystal X-ray diffraction.[12] Furthermore, the absolute configuration that was originally assigned by the method of Horeau[5] has been confirmed by several unambiguous total syntheses based on chiral starting materials.[13] For the synthesis of **7a**, the cis-1,2-diol moiety in swainsonine was protected as the acetonide (**8**, 85%). The 8-hydroxyl group was then removed by the method of Barton[14] by converting **8** to the *S*-methyl dithiocarbonate **9**, which was reduced with tributyltin hydride to give acetonide **10** in 62% yield (Scheme III). The acetonide was hydrolyzed to diol **7a**, which was converted to diacetate **7b**. In the NMR spectrum of **7b**, the key vicinal coupling constant, $J_{1,8a}$, is 4.9 Hz, comparable to that in swainsonine. The spectroscopic and chromatographic properties of **7b** were clearly distinct from those of the diacetate of the diol metabolite; hence a synthesis of **6a** and its derivatives was undertaken.

As we were completing our synthesis of **6a** and **6b**, Colegate et al. published a synthesis of **6a**; the reported spectra of **6a** and its diacetate (**6b**) corresponded well with those of the metabolite and its diacetate.[15] Their synthesis involved alkylation of the lithium salt[16,17] of *N*-(meth-

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(13) Ali, M. H.; Hough, L.; Richardson, A. C. *J. Chem. Soc., Chem. Commun.* **1984**, 447–448. Fleet, G. W. J.; Gough, M. J.; Smith, P. W. *Tetrahedron Lett.* **1984**, *25*, 1853–1856; *Carbohydr. Res.* **1985**, *136*, 225–240. Suami, T.; Tadano, K.-i.; Iimura, Y. *Chem. Lett.* **1984**, 513–516; *Carbohydr. Res.* **1985**, *136*, 67–76. Yasuda, N.; Tsutsumi, H.; Takaya, T. *Chem. Lett.* **1984**, 1201–1204. Adams, C. E.; Walker, F. J.; Sharpless, K. B. *J. Org. Chem.* **1985**, *50*, 420–422.

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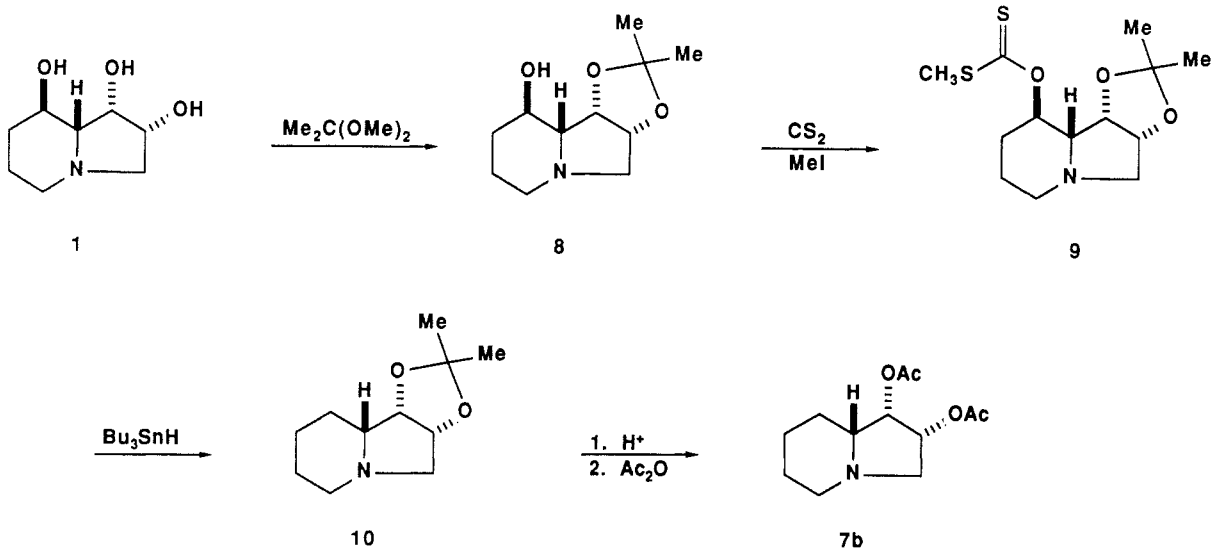
(15) Colegate, S. M.; Dorling, P. R.; Huxtable, C. R. *Aust. J. Chem.* **1984**, *37*, 1503–1509.

Scheme III



oxycarbonyl)-3-pyrroline with 1,4-dibromobutane to give bromide **11**, which was then oxidized with OsO_4/H_2O_2 to form pyrrolidinediol **12**. The amine resulting from cleavage of the urethane with trimethylsilyl iodide cyclized spontaneously to give a single diol, assigned as **6a**. Their assignment of the stereochemistry of **6a** is based on mechanistic considerations, i.e., the anticipated attack by OsO_4 on the less hindered face of the double bond in **11**. Previous studies of the reaction of OsO_4 with *N*-Cbz-3,4-didehydro-prolinamide had given exclusively the 2,3-trans, 3,4-cis 3,4-diol.[18] However, it should be noted that the reaction of didehydroproline itself with $KMnO_4$ gave an equal mixture of the 2,3-cis, 3,4-cis and 2,3-trans, 3,4-cis diols.[18] Colegate's stereochemical assignment is supported by the 8.2-Hz coupling constant observed between H-1 and H-8a, which compares favorably with the 8.0-Hz coupling in the spectrum of the pyrrolizidine alkaloid croalbinecine for similar protons (H-1 and H-7a) known to be trans.[19]

We chose a closely related route for our preparation of **6a**. Glycolation of pyrroline **11** with OsO_4/*N*-methylmorpholine *N*-oxide gave only diol **12**. An attempt to deprotect **12** with methyllithium, as described by Macdonald,[17] gave mainly hexahydrooxepane **13** arising by intramolecular displacement of the Ω-bromine by alkoxide. Even after protection of the diol as the acetonide (**14**), methyllithium still failed to remove the methoxycarbonyl group satisfactorily and the group was ultimately cleaved from **14** by treatment with trimethylsilyl iodide. The liberated amine cyclized spontaneously to give indolizidine **6c**, which was converted to diacetate **6b** by treatment with acetic anhydride. Synthetic **6b** and the diacetate of the natural diol were compared by chromatography and by NMR and found to be identical. It should be noted that our unambiguous synthesis of diol **7a** from swainsonine confirms the stereochemical assignment of **6a–c**.

The biosynthetic relationship of diol **6a** to swainsonine was examined by refeeding the [^{3}H] diol **6a**, which had been isolated from the fungus. A very high level (45%) of incorporation of radioactivity into swainsonine was found. In view of the fact that the diol moiety is cis in both **6a** and swainsonine, it is reasonably secure that the absolute configurations of the two compounds are identical at these positions. Therefore, we assign the absolute configuration of **6a** as 1*S*,2*R*,8a*S*.

The formation of **6a** coupled with its efficient conversion to swainsonine provides strong evidence for **6a** being on the pathway to swainsonine. However, it may not be an obligatory intermediate but only a byproduct of the true pathway in view of the fact that production of **6a** is enhanced when the swainsonine pathway is flooded with exogenous 1-hydroxyindolizidine. Details of the conversion of **6a** to swainsonine remain obscure. Although iminium ions are probably involved in the epimerization at 8a since isotopic label is lost from that position, many scenarios can be proposed. Three involving iminium ions in the epimerization are shown in Scheme IV: (a) diol **6a** could epimerize to **7a** via iminium ion **15** and then be hydroxylated to give **1**; (b) **15** might be hydroxylated to give **16** and then reduced to give **1**; or (c) **6a** might be hydroxylated first to give *epi*-swainsonine (**17**) and then oxidized to iminium ion **16** and reduced to **1**.[20]

We studied the first of these possibilities using the authentic sample of diol **7a** which had been prepared from swainsonine. A careful but unsuccessful search was made for **7a** in cultures of *R. leguminicola* by using capillary gas/liquid chromatography and GC–mass spectrometry. Using conditions under which authentic samples of diacetates **6b** and **7b** are well-resolved (t_R 21.7 and 22.5 min), only **6b** could be seen in unfractionated mixtures of acetylated metabolites. A similar result was obtained by analytical ion-exchange chromatography of the free diols **6a** and **7a** with ^{3}H analysis. Further studies of the conversion of **6a** to **1** are in progress.

Experimental Section

General Procedures. NMR spectra were recorded on JEOL FX-90Q and Bruker AM-400 spectrometers (^{1}H at 90 and 400 MHz, respectively). Low resolution mass spectra were recorded

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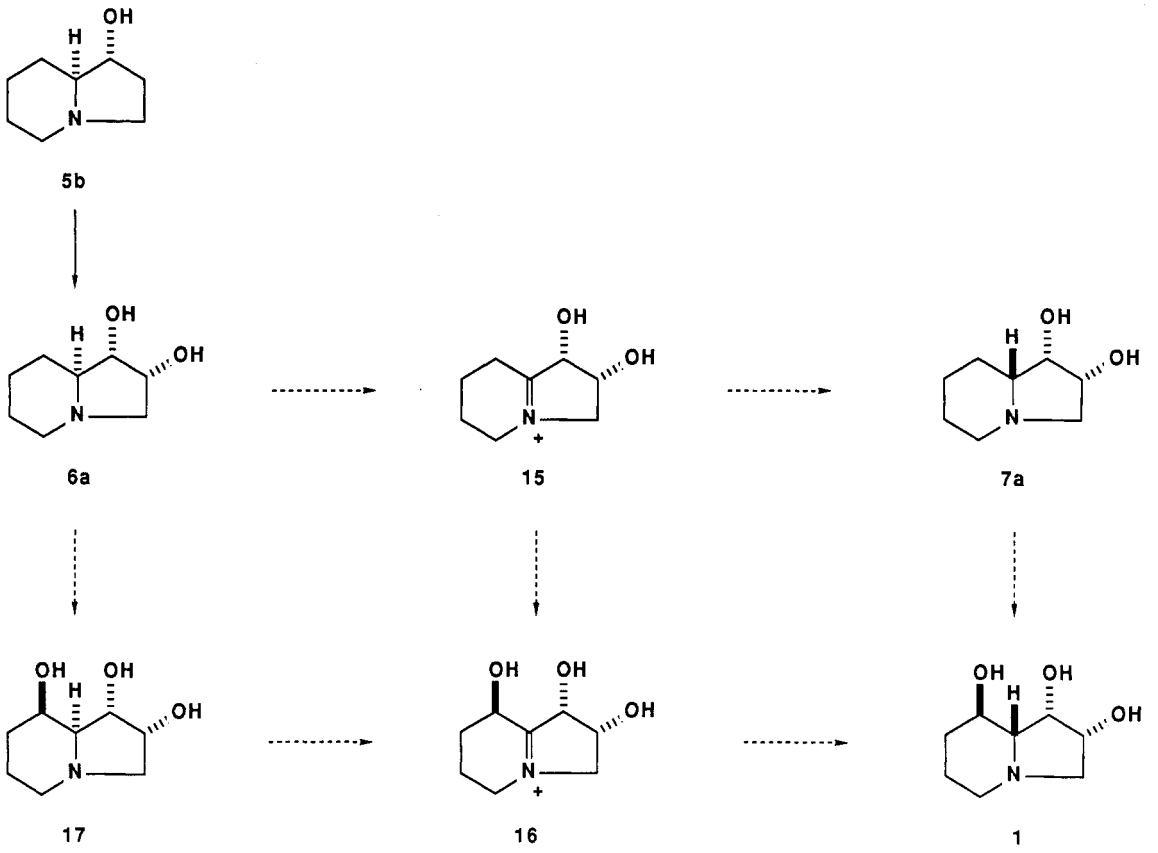
(20) A referee has suggested that epimerization might occur by hydride shift from C-1 to C-8a in iminium ion **15** or **16**. Thus, [1-^{3}H]-**6a** would yield [8a-^{3}H]-**1**. This proposal cannot presently be evaluated because we lack methods for degrading swainsonine to establish the location of the ^{3}H label or for preparing sufficient quantities of [1-^{2}H]-**6a** with high isotopic content to employ in a feeding experiment. However, the question will be dealt with in a future communication.

Scheme IV



on LKB-9000 and Ribermag R-1010B spectrometers and high resolution mass spectra on a VG Micromass 7070E spectrometer. Radioactivity was measured on a Beckman LS-100 liquid scintillation counter using [^{3}H]toluene as an internal standard. During the workup of reactions, extracts were routinely dried over $MgSO_4$ prior to evaporation on a Buchi rotavapor at aspirator pressure. Chromatographic solvents are expressed as percent by volume of the first component in the solvent mixture.

Growth Conditions for the Fungus. *Rhizoctonia leguminicola* Gough et E. S. Elliot (ATCC #26280) was obtained on agar slants from the American Type Culture Collections, Rockville, MD, and stored on slants of filtered red clover hay infusion (10% w/v) hardened with 1.5% Bacto-Agar (Difco). Inocula were maintained on sterile, filtered red clover hay infusion (240 mL, 10% w/v) in 1-L Roux bottles at 20–25 °C. At 3–4-week intervals subcultures were prepared by blending a mycelial mat with 100 mL of sterile water and transferring 10-mL aliquots of the resulting suspension to fresh hay infusion in Roux bottles. A fresh culture line was begun from a slant every 3–4 months.

Metabolite Production. The growth conditions described by Clevenstine et al.[9] were employed with the following modification: the red clover hay infusion medium was replaced by Czapek Dox medium containing 0.3% Difco Bacto yeast extract. Diol **6a** was produced in a mat with media supplemented on the day of inoculation with 0.49 mmol of [1-^{3}H]-**5a,b**[11] (5.69 mCi/mmol). The mycelium was harvested on day 14 and extracted overnight by using a Soxhlet apparatus. Diol **6a** was separated from swainsonine and **5a,b** on a Biorad AG-50-X8 column with a 0.1 M $NaHCO_3$/0.1 M NaOH gradient. The sequence of elution on this column is pipecolic acid, swainsonine, diol **6a**, trans alcohol **5b**, deacetyl slaframine, cis alcohol **5a**. The yields of the endogenous alkaloids per mat were estimated to be 7.0 mg of swainsonine, 1.7 mg of deacetyl slaframine, and 0.3 mg of **6a**.

Diol **6a** could also be isolated from fermentations that had not been supplemented with substantial quantities of **5a,b**. When only tracer quantities of [1-^{3}H]-**5a,b** were added (to aid pooling of chromatography fractions), several hundred mats were required to produce sufficient amounts of endogenous **6a** for spectroscopic characterization. Diol **6a**: MS (EI), m/z 157, 97; ^{13}C NMR (acetone-d_6) δ 75.0, 67.3, 66.9, 60.9, 52.8, 28.2, 24.8, 23.5. Treatment of **6a** with Ac_2O by the procedure previously used[5] for acetylation of swainsonine gave diacetate **6b**: ^{1}H NMR ($CDCl_3$) δ 5.24 (1 H, ddd, $J_{2,1}$ = 7 Hz, $J_{2,3}$ = 7 Hz, $J_{2,3'}$ = 5 Hz, H-2), 4.77 (1 H, dd, $J_{1,2}$ = 7 Hz, $J_{1,8a}$ = 9 Hz, H-1), 3.60 (1 H, dd, $J_{3,3'}$ = 10 Hz, $J_{3,2}$ = 7 Hz, H-3 [cis to H-2]), 3.03 (1 H, dd, $J_{5,5'}$ = 11 Hz, $J_{5,6}$ = 2 Hz, H-5$_{eq}$), 2.4–1.0 (all remaining protons); CI–MS, m/z (relative intensity) 242 (M + H, 100), 240 (16), 182 (56), 181 (9), 123 (9), 122 (96); exact mass (EI), m/z 241.1318, calcd for $C_{12}H_{19}NO_4$ 241.1315.

Swainsonine Acetonide (8). Following the procedure of Schneider et al.,[5] a mixture of swainsonine (0.20 g, 1.16 mmol), 2,2-dimethoxypropane (2 mL), acetone (18 mL), and *p*-toluenesulfonic acid (2.0 g) was stored at 5 °C for 2 days. Workup, including chromatography on silica gel (10 g, 10% MeOH/CH_2Cl_2), gave 85% of acetonide **8**: ^{1}H NMR ($CDCl_3$) δ 4.72 (1 H, dd, $J_{1,2}$ = 6.3 Hz, $J_{1,8a}$ = 4.1 Hz, H-1), 4.63 (1 H, dd, $J_{2,1}$ = 6.3 Hz, $J_{2,3'}$ = 4.0 Hz, H-2), 3.86 (1 H, ddd, $J_{8,8a}$ = 6.3 Hz, $J_{8,7ax}$ = 8.4 Hz, $J_{8,7eq}$ = 4.2 Hz, H-8), 3.16 (1 H, d, $J_{3,3'}$ = 10.7 Hz, H-3), 3.00 (1 H, ddd, $J_{5eq,5ax}$ = 9.7 Hz, $J_{5eq,6eq}$ = 3.0 Hz, $J_{5eq,6ax}$ = 3.0 Hz, H-5$_{eq}$), 2.15 (1 H, br d, $J_{3,3'}$ = 10.7 Hz, $J_{3',2}$ = 4.0 Hz, H-3'), 1.96 (1 H, m, $J_{7eq,7ax}$ = 11.8 Hz, $J_{7eq,8}$ = 4.2 Hz, H-7$_{eq}$), 1.86 (1 H, br m, H-5$_{ax}$), 1.66 (2 H, br m, H-8a and H-6$_{eq}$), 1.53 (3 H, s, CH_3), 1.34 (3 H, s, CH_3), 1.25 (2 H, br m, H-6$_{ax}$ and H-7$_{ax}$).

Acetonide 10 Prepared from 8. NaOH (1 mL, 40% aqueous) was added dropwise with cooling to a mixture of acetonide **8** (0.070 g, 0.30 mmol), CS_2 (0.09 g, 1.2 mmol), $(n\text{-Bu})_4NHSO_4$ (0.12 g, 0.35 mmol), and benzene (1 mL). After 15 min, iodomethane (0.06 g, 0.40 mmol) was added, and after an additional 15 min the mixture was poured into ice/Et_2O and extracted with Et_2O. The combined extracts were evaporated and the residue was chromatographed on silica gel (10 g, CH_2Cl_2) to give 55% of the dithiocarbonate ester **9** as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 5.60 (1 H, m), 4.58 (2 H, br m), 3.22 (1 H, d), 2.56 (3 H, s), 1.47 (3 H, s), 1.29 (3 H, s), 3.2–1.2 (8 H, m); ^{13}C ($CDCl_3$) δ 212.4, 111.7, 78.9, 78.8, 78.3, 70.3, 60.2, 51.3, 28.9, 26.1, 25.3, 23.7, 18.9; IR (neat) cm^{-1} 2950, 2800, 1475, 1385, 1240, 1220, 1060; EI–MS, m/z (relative intensity) 288 (M – CH_3, 6), 196 (18), 195 (100).

A solution of $(n\text{-Bu})_3SnH$ (0.065 mL, 0.2 mmol) in xylene (3 mL) was slowly added to a refluxing mixture of **9** (0.050 g, 0.15 mmol) and dry, degassed xylene (5 mL) under N_2. After 48 h at reflux, the mixture was cooled and poured into Et_2O/dilute HCl. The ether layer was discarded; the aqueous layer was taken to

pH 10 (K_2CO_3) and extracted with CH_2Cl_2. The organic extract was evaporated and the residue chromatographed on silica gel (10 g, 5% MeOH/CH_2Cl_2) to give 62% of acetonide **10** as a clear oil and 10% of diol **7a**. Compound **10**: ^1H NMR ($CDCl_3$) δ 4.57 (1 H, dd, $J_{1,8a}$ = 4.2 Hz, $J_{1,2}$ = 6.3 Hz, H-1), 4.49 (1 H, br dd, $J_{2,1}$ = 6.3 Hz, $J_{2,3'}$ = 3.2 Hz, $J_{2,3}$ = 0 Hz, H-2), 3.10 (1 H, br d, $J_{3,2}$ = 0 Hz, $J_{3,3'}$ = 10.7 Hz, H-3), 3.10 (1 H, br d, $J_{5eq,5ax}$ = 10.7 Hz, H-5_{eq}), 2.13 (1 H, dd, $J_{3',2}$ = 3.2 Hz, $J_{3',3}$ = 10.7 Hz, H-3'), 1.65 (1 H, m, $J_{8a,1}$ = 4.2 Hz, H-8a), 1.86–1.30 (7 H, m, H-5_{ax}, H-6_{eq}, H-6_{ax}, H-7_{eq}, H-7_{ax}, H-8_{eq}, H-8_{ax}), 1.52 (3 H, s, CH_3), 1.32 (3 H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 111.0 (s), 81.2 (d), 78.3 (d), 68.0 (d), 60.4 (t), 53.0 (t), 26.0 (q), 25.2 (t), 24.9 (2×, q and t), 24.2 (t).

Diacetate 7b from 10. Acetonide **10** (0.020 g, 0.10 mmol) was treated with 2 M HCl (6 mL) for 6 h at 80 °C. The mixture was lyophilized and the residue (the hydrochloride of **7a**) was treated with Ac_2O for 1 h at 60 °C. Water was added and the mixture was concentrated in vacuo. The residue was taken to pH 10 (K_2CO_3) and extracted with CH_2Cl_2 The extract was concentrated and chromatographed on neutral alumina (10 g, 30% EtOAc/hexane) to give 83% of diacetate **7b**: ^1H NMR (acetone-d_6) δ 5.25 (1 H, ddd, $J_{1,8a}$ = 4.9 Hz, $J_{1,2}$ = 6.5 Hz, H-1), 5.15 (1 H, ddd, $J_{2,1}$ = 6.5 Hz, $J_{2,3}$ = 1.5 Hz, $J_{2,3'}$ = 7.1 Hz, H-2), 2.98 (1 H, br d, $J_{5eq,5ax}$ = 10.5 Hz, H-5_{eq}), 2.95 (1 H, dd, $J_{3,2}$ = 1.5 Hz, $J_{3,3'}$ = 11.0 Hz, H-3), 2.37 (1 H, dd, $J_{3',2}$ = 7.1 Hz, $J_{3',3}$ = 11.0 Hz, H-3'), 2.07 (1 H, m, H-6_{eq}), 2.04 (1 H, m, H-8a), 2.00 (3 H, s, CH_3CO), 1.97 (3 H, s, CH_3CO), 1.77 (1 H, m, H-5_{ax}), 1.75 (1 H, m, H-8_{eq}), 1.55 (1 H, m, H-7_{eq}), 1.45 (1 H, m, H-6_{ax}), 1.39 (1 H, m, H-8_{ax}), 1.21 (1 H, m, H-7_{ax}); ^{13}C NMR (acetone-d_6) δ 170.4 (2×), 73.4, 71.4, 66.9, 59.8, 53.5, 25.7, 25.6, 24.5, 20.6, 20.4; CI–MS, m/z (relative intensity) 242 (M + H, 100), 240 (13), 182 (7), 181 (5), 123 (7), 122 (70); EI–MS, m/z (relative intensity) 241 (M, 100), 239 (13), 181 (7), 121 (70); exact mass (EI), m/z 241.1318, calcd for $C_{12}H_{19}NO_4$ 241.1315.

1-(Methoxycarbonyl)-2-(4-bromobutyl)-3-pyrroline (11). To a mixture of *N*-carbomethoxy-3-pyrroline[15] (1.46 g, 0.011 mol), 1,4-dibromobutane (11.0 g, 0.051 mol), and THF (25 mL) under N_2 and at −78 °C was added a solution of LDA (0.021 mol) in THF (25 mL) at −78 °C over 15 min using a bridging cannula. The reaction was quenched after 45 min at 0 °C by addition of ice/dilute HCl. The reaction mixture was concentrated to remove THF, acidified to pH 5.0, and extracted with CH_2Cl_2. The extract was concentrated and chromatographed on silica gel (10 g, CH_2Cl_2); the resulting oil was distilled bulb-to-bulb (40 °C/0.05 mm) and then flash chromatographed (30% EtOAc/hexane) to give 60% of **11** as a colorless oil: ^1H NMR ($CDCl_3$) major rotamer δ 5.76 (2 H, br s), 4.58 (1 H, br m), 4.14 (2 H, br m), 3.72 (3 H, s), 3.40 (2 H, t, J = 7.2 Hz), 2.00–1.30 (6 H, m); ^{13}C NMR ($CDCl_3$) major rotamer δ 155.2, 129.7, 125.2, 64.4, 53.6, 52.1, 33.4, 32.7 (2×), 22.9; IR (neat) cm^{-1} 2950, 2860, 1710 (s), 1450, 1385, 1190, 1120, 1110; EI–MS, m/z (relative intensity) 263 (M, 68), 261 (M, 79), 181 (77), 125 (100).

Oxidation of 11 with OsO_4 To Give 12. *N*-Methylmorpholine *N*-oxide (0.70 g, 0.006 mol) and OsO_4 (9 mg in 0.4 mL of *tert*-butyl alcohol) were added to a solution of pyrroline **11** (0.88 g, 0.0034 mol) in acetone (9 mL) and H_2O (4 mL) at 0 °C. The mixture was stirred at room temperature for 16 h, then quenched with a slurry of $NaHSO_3$ (0.02 g) and magnesium trisilicate (0.24 g) in H_2O (2 mL), and filtered through Celite. The solution was concentrated and extracted with EtOAc. The organic solution was evaporated and chromatographed on silica gel (10 g, 5% MeOH/CH_2Cl_2) to give a quantitative yield of diol **12** as a colorless oil: ^1H NMR ($CDCl_3$) major rotamer δ 4.27 (1 H, br m), 3.93 (2 H, br m), 3.69 (3 H, s), 4.0–3.6 (2 H very br, hydroxyls), 3.51 (2 H, m), 3.43 (2 H, t, J = 6.3 Hz), 2.04–1.36 (6 H, m); ^{13}C NMR ($CDCl_3$) major rotamer δ 156.1 (s), 75.2 (d), 69.9 (s), 63.2 (d), 52.5 (q), 50.5 (t), 33.4 (t), 32.3 (t), 31.2 (t), 24.0 (t); IR (neat) cm^{-1} 3400 (br), 2950, 1680, 1460, 1390, 1190, 1095; EI–MS, m/z (relative intensity) 297 (M, 80), 295 (M, 86), 265 (57), 263 (56), 215 (98), 159 (100).

Acetonide 14. A mixture of diol **12** (2.9 g, 0.010 mol), 2,2-dimethoxypropane (7 mL), acetone (35 mL), and H_2SO_4 (1 drop) was stored at 5 °C for 2 days, neutralized with 10% aqueous $NaHCO_3$ (20 mL), concentrated, and extracted with CH_2Cl_2. The organic extract was concentrated and chromatographed on silica gel (20 g, EtOAc/hexane gradient) to give acetonide **14** (94%) as a light yellow oil: ^1H NMR ($CDCl_3$) δ 4.72 (1 H, t, J = 5.4 Hz), 4.41 (1 H, d, J = 5.4 Hz), 4.03 (2 H, br m), 3.71 (3 H, s), 3.41 (2 H, t, J = 6.3 Hz), 3.34 (1 H, m), 1.90–1.49 (6 H, br m), 1.43 (3 H, s), 1.30 (3 H, s); ^{13}C NMR ($CDCl_3$) δ 155.5, 111.7, 84.0, 79.0, 63.3, 52.4, 51.2, 33.1, 32.1, 30.1, 26.8, 24.8, 24.2; IR (neat) cm^{-1} 2990, 2945, 2860, 1710, 1455, 1395, 1210; EI–MS, m/z (relative intensity) 337 (M, 60), 335 (M, 66), 305 (36), 303 (35), 279 (36), 277 (34), 255 (100), 199 (58).

Carbamate Cleavage on 14 by Me_3SiI. A mixture of acetonide **14** (0.5 g, 1.5 mmol) and Me_3SiI (0.26 mL, 1.8 mmol) in dry $CHCl_3$ (1 mL) was refluxed for 3 h and evaporated. The residue was taken up in EtOH (10 mL) containing K_2CO_3 (0.5 g, 3.6 mmol) and heated at 60 °C for 2 days. The mixture was cooled and CH_2Cl_2 (50 mL) was added. Salts were removed by two cycles of filtering and concentrating to dryness. Chromatography on silica gel (10 g, 5% MeOH/CH_2Cl_2) gave acetonide **6c** (83%) as a light yellow oil which solidified at −10 °C: ^1H NMR ($CDCl_3$) δ 4.68 (ddd, 1 H, H-2, $J_{2,1}$ = 7.5 Hz, $J_{2,3}$ = 7.0 Hz, $J_{2,3'}$ = 5.0 Hz), 4.19 (dd, 1 H, $J_{1,2}$ = 7.5 Hz, $J_{1,8a}$ = 6.2 Hz), 3.36 (dd, 1 H, H-3, $J_{3,2}$ = 7.0 Hz, $J_{3,3'}$ = 9.5 Hz), 2.97 (br d, 1 H, H-5_{eq}, $J_{5eq,5ax}$ = 11 Hz), 2.31 (dd, 1 H, H-3', $J_{3',2}$ = 5 Hz, $J_{3',3}$ = 9.5 Hz), 2.14 (m, 1 H, H-5_{ax}), 2.00 (m, 1 H, H-8_{eq}), ~1.94 (m, 1 H, H-8a), 1.80 (br m, 1 H, H-6_{eq}), 1.58 (br d, 1 H, H-7_{eq}), 1.45 (m, 1 H, H-6_{ax}), 1.22 (m, 2 H, H-7_{ax}, H-8_{ax}), 1.51 (s, 3 H, CH_3), 1.33 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 113.8 (s), 84.56 (d), 77.49 (d), 68.85 (d), 60.10 (t), 52.54 (t), 28.97 (t), 27.21 (q), 25.16 (q), 24.78 (t), 24.00 (t); MS, m/z (relative intensity) 197 (M, 100), 139 (50), 96 (39).

Conversion of 6c to Diacetate 6b. Acetonide **6c** (0.05 g, 0.2 mmol) was treated with 2 M HCl (10 mL) for 16 h at 80 °C. The solution was washed with CH_2Cl_2 and evaporated to dryness. The residue (the hydrochloride of **6a**) was treated with Ac_2O (20 mL) for 3 h at reflux. The mixture was cooled to 0 °C, H_2O (10 mL) was slowly added, and the volume was reduced in vacuo. Water was added followed by $NaHCO_3$ until the solution became basic. Extraction (CH_2Cl_2) gave 70% of diacetate **6b**: ^1H NMR ($CDCl_3$) δ 5.23 (1 H, ddd, $J_{2,1}$ = 7.2 Hz, $J_{2,3}$ = 7.0 Hz, $J_{2,3'}$ = 5.3 Hz, H-2), 4.75 (1 H, dd, $J_{1,8a}$ = 8.8 Hz, $J_{1,2}$ = 7.2 Hz, H-1), 3.58 (1 H, dd, $J_{3,2}$ = 7.0 Hz, $J_{3,3'}$ = 10.0 Hz, H-3), 3.03 (1 H, br d, $J_{5eq,5ax}$ = 10.8 Hz, H-5_{eq}), 2.22 (1 H, dd, $J_{3',2}$ = 5.3 Hz, H-3'), 2.13 (1 H, ddd, $J_{5ax,5eq}$ = 10.8 Hz, $J_{5ax,6eq}$ = 1.7 Hz, $J_{5ax,6ax}$ = 4.0 Hz, H-5_{ax}), 2.08 (1 H, m, H-8a), 2.06 (3 H, s, CH_3CO), 2.04 (3 H, s, CH_3CO), 1.82 (2 H, m), 1.61 (2 H, m), 1.25 (2 H, m); ^{13}C NMR ($CDCl_3$) δ 169.7 (2×, s), 74.91 (d), 68.22 (d), 64.89 (d), 59.18 (t), 52.51 (t), 28.54 (t), 24.94 (t), 23.50 (t), 20.39 (q), 20.25 (q); IR (KBr) cm^{-1} 2945, 2800, 1750, 1440, 1370, 1250, 1065; CI–MS, m/z (relative intensity) 242 (M + H, 100), 240 (10), 182 (32), 181 (7), 123 (8), 122 (84); EI–MS, m/z (relative intensity) 241 (M, 100), 239 (10), 181 (18), 121 (51).

Feeding Experiment with [1-^{3}H]-6a. The liquid was decanted from a 5-day-old mat produced on Czapek-Dox medium. Radioactive [1-^{3}H]-**6a** (0.26 μCi) dissolved in 50 mL of sterile water was injected under the mat. After 48 h, the mat was harvested and extracted with ethanol for 16 h using a Soxhlet apparatus. The extract was separated by ion-exchange chromatography on AG-50 resin. The swainsonine fraction contained 0.12 μCi of ^{3}H, representing 45% incorporation of **6a** into the alkaloid. The yield of swainsonine was 3 mg as determined by α-mannosidase assay.[4,21]

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