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MAMANINE AND POHAKULINE, TWO UNPRECEDENTED QUINOLIZIDINE ALKALOIDS FROM *SOPHORA CHRYSOPHYLLA*[1]

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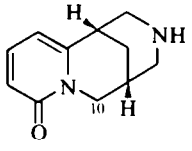
Abstract—From the bark of the endemic Hawaiian tree *Sophora chrysophylla* Seem. we have isolated two unprecedented quinolizidine alkaloids, mamanine (**4**) and pohakuline (**5**). Both bases are 1-hydroxymethylenequinolizidines which are linked at C-3 to α-pyridone or α-piperidone moieties. The alkaloids may be intermediates in a heretofore unknown biogenetic pathway of *Sophora* alkaloids.

Our continuing research on endemic Hawaiian plant constituents led us to an investigation of the bark alkaloids of *Sophora chrysophylla* Seem. (Leguminosae), which is the sole Hawaiian *Sophora* species. It occurs on most of the principal islands from sea level, where it is a shrub, to an elevation of 3000 m, where it is a large tree (Hawaiian name, *mamane*) and grows to a height of 15 m.[2] Briggs and Russell[3] had studied *mamane* seeds, from which they isolated the widely distributed alkaloids (−)cytisine (**1**) and (−)anagyrine (**2**), both typical representatives of *Sophora* species, and also an incompletely



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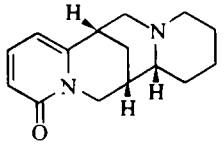

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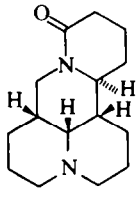

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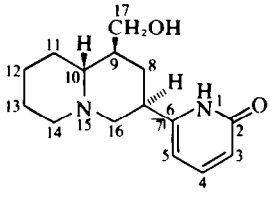
characterized base, sophochrysine. From the bark of *S. chrysophylla*, which contained alkaloids only when the tree was flowering, we isolated cytisine (**1**) and matrine (**3**) and two new alkaloids which we have named mamanine (**4**) and pohakuline¶ (**5**) and which are the subject of this report. The novel structure of these two *Sophora* alkaloids poses an interesting biogenetic question.



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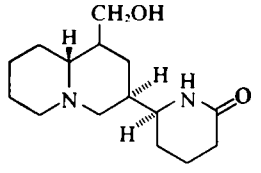

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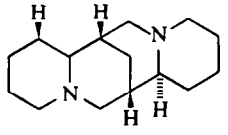

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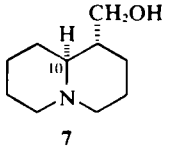
Biosynthesis of quinolizidine alkaloids has been studied extensively, most recently by Nowacki and Waller.[4a–c] All evidence to-date indicates that quinolizidine alkaloids in the Leguminosae are elaborated from lysine, first toward saturated oxygen-free tetracycles such as (+)sparteine (**6**), hence into tetracyclic derivatives of various oxidation levels, e.g. anagyrine (**2**), which eventually are degraded to the tricyclic alkaloids of which cytisine (**1**) is a representative. Our discovery in *S. chrysophylla* of the two unbridged tricyclic quinolizidine alkaloids mamanine (**4**) and pohakuline (**5**) may signify yet another bypath of the established scheme or else may represent a new potential biosynthetic route toward tetracyclic alkaloids: mamanine (**4**) and pohakuline (**5**) may arise by ring scission of a suitable tetracyclic precursor or by addition of a C-10 lupinine isomer (**7**) to piperideine (**8**) or an oxidized equivalent. Until pertinent biosynthetic experi-



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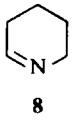

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†From the M.S. thesis of M.M.K., University of Hawaii (1974).
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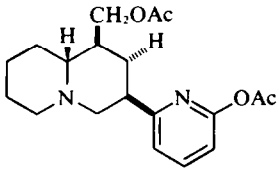
¶We collected the bark in the Pohakuloa area, island of Hawaii, at an elevation of 2000 m.

ments have been carried out, both pathways appear plausible.

Mamanine. Methanol extraction of ground dried *mamane* bark, followed by acid leaching of the residue and alumina chromatography led to the isolation of matrine (**3**), cytisine (**1**), pohakuline (**5**) and mamanine (**4**) as a dihydrate, m.p. 171–172°, after first melting ~100° and resolidifying. It formed a crystalline cyclohexylsulfamate, m.p. 245–252°. Combustion data pointed to a composition of $C_{16}H_{24}N_2O_2$ for the free base, but subsequent mass spectral and CMR measurements secured a formulation of $C_{15}H_{22}N_2O_2$. UV[5] and IR data suggested an α-pyridone moiety, but absence of a complex PMR signal near δ 4 normally assigned to the C-10 protons of cytisine (**1**)[7] indicated that neither cytisine (**1**) nor anagyrine (**2**) were adequate models for mamanine (**4**). A low-field PMR scan of mamanine (**4**) in DMSO-d_6 revealed a broad peak at δ 11·53 integrating for nearly one proton that we assigned to an imino proton of an α-pyridone in fair agreement with a published value of δ 12·05 ($CDCl_3$) for such a proton.[8]

The hydroxymethylene function in mamanine (**4**) gave rise to unequivocal IR and NMR signals as well as to characteristic mass spectral peaks (M–OH, M–H_2O, M–CH_2OH).

Acetylation of mamanine (**4**) furnished a diacetate (**9**)



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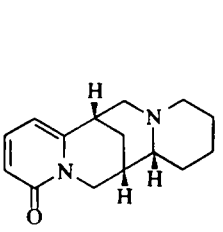
with two sharp 3H singlets at δ 2·09 and 2·38 in its PMR spectrum. The high field signal is readily assigned to the primary alcohol acetate, but the signal at δ 2·38 is at too low a field for an N-acetyl or an alcoholic O-acetyl group. This chemical shift, however, is comparable with that of a phenolic acetate, e.g. 2-acetoxyacetophenone, which resonantes at δ 2·47.[9] Production of a di-O-acetyl derivative, in turn, further confirms presence in mamanine of an N-unsubstituted α-pyridone, which characteristically is acetylated as the lactim rather than the lactam tautomer.[10]

Establishment of α-pyridone and hydroxymethylene functions coupled with common occurrence of quinolizidine alkaloids in the genus *Sophora*, suggests a disubstituted quinolizidine nucleus for the unaccounted $C_9H_{15}N$ part of mamanine (**4**). Direct IR and PMR evidence for simple quinolizidines is difficult: they possess nondescript IR and complex PMR spectra. Bohlmann *et al.*[11] succeeded in assigning chemical shifts to only five of the seventeen quinolizidine protons. Biogenetic considerations favor a mamanine (**4**) skeleton, which bears relation to anagyrine (**2**) or thermopsine (**10**), or alternately **11**, which is reminiscent of the known sophoramine (**12**) or its C-16 epimer isosophoramine. Mass and CMR spectral data unambiguously favored the mamanine (**4**) structure.

Both structures **4** and **11** are expected under electron impact to lose allyl alcohol and produce a stable fragment **a** or **b** (base peak *m/e* 204), in full analogy with the fragmentation of lupinine (**13**) or its C-1 epimer epilupinine.[12] Further fragmentation of the stable *m/e* 204 ion into fragments observed at *m/e* 122, 121, 84 and 83,



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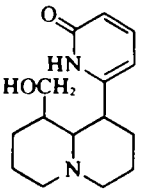

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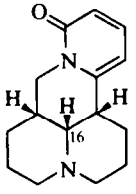

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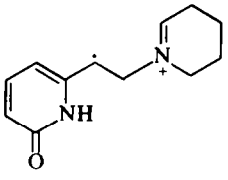
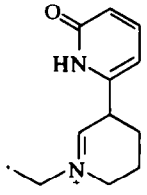


a



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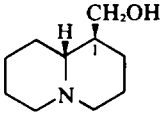

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and confirmed by metastable peaks, is readily rationalized by **a** but not by **b**. The fragmentation pattern of the diacetate (**9**) fully confirms structure **4**.

Finally, complete assignment of all CMR data can be made for mamanine (**4**) in analogy with published values for α-pyridone[13] and quinolizidine.[14]

Confirmation of structure **4** for mamanine and relative stereochemical assignment was achieved by X-ray diffraction studies.

Pohakuline. Pohakuline (**5**) was present in *S. chrysophylla* bark in very small amount (0·0034%). It crystallized from acetone, m.p. 170–171°. Combustion data, later confirmed by mass spectral measurements, indicated a composition of $C_{15}H_{26}N_2O_2$, differing from mamanine (**4**) by four H atoms. It formed an amorphous chloraurate and could be reduced by LAH to a base $C_{15}H_{28}N_2O$, corresponding to transformation of a lactam to an amine.

All spectral data of pohakuline (**5**) are consistent with an alkaloid that contains hydroxymethylene, quinolizidine and α-piperidone functions. Biogenetic considerations are equally compatible with a tetrahydromamanine (**4**) skeleton or a tetrahydro analog of **11**. Our small supply (10 mg) of pohakuline (**5**) precluded distinction between these two types by chemical or spectral means. Structure **5** was established by X-ray diffraction.

Mamanine (**4**), $C_{15}H_{22}N_2O_2$, crystallized in the orthorhombic space group $P2_12_12_1$ with $a = 11{\cdot}088(1)$ Å $b = 10{\cdot}733(1)$ Å and $c = 27{\cdot}226(3)$ Å with two molecules of mamanine and four molecules of H_2O in the asymmetric unit for a calculated density of 1·15 g/cm^3. A total of 2384 diffraction maxima with $\theta \leq 57°$ were measured using graphite monochromated Cu radiation (1·5418 Å) and an automatic four-circle diffractometer. After correction for Lorentz, polarization, and background effects a total of 2100 (88%) were judged observed using $F_o^2 \geq 3\sigma(F_o^2)$. The three dimensional crystal structure was solved routinely using a direct methods approach.[15] Full-matrix least squares refinements smoothly lowered the conventional discrepancy index to 0·056 using anisotropic temperature factors for non-H atoms and isotropic temperature factors for H atoms.[16] Figure 1 is a computer-generated drawing of the final model of mamanine (**4**).[17] Tables 1 and 2 contain the bond distances and bond angles. All bond distances and angles are in close agreement with commonly accepted values for the two independent molecules.

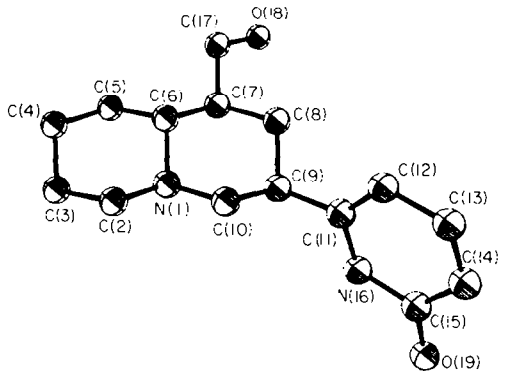

Fig. 1. A computer generated perspective drawing of mamanine (**4**). Hydrogens are not shown and no absolute stereochemistry is implied.

With reference to Fig. 1 it is clear that rings A and B which are joined by a trans ring junction both adopt a chair conformation. In addition the hydroxymethylene and the α-pyridone ring occupy equatorial positions on ring B. Since the asymmetric unit contains two molecules of mamanine (**4**) and four molecules of H_2O there is presumed to be a considerable amount of H-bonding in the crystal structure. As we were unable to find satisfactory positions for the H_2O hydrogens, no results concerning the H- bonding involving H_2O are available. However, the two pyridone moieties in the asymmetric unit are connected by two intermolecular H-bonds. One H-bond has the parameters N(16)–H(16) 0·87 Å; O(19′)–H(16) 1·92 Å; N(16)–O(19′) 2·78; with an angle of 169·8° for N(16)–O(19′). The other bond has distances of N(16′)–H(16′) 1·00 Å; O(19)–H(16′) 1·82 Å; N(16′)–O(19) 2·81 Å and the angle N(16′)–H(16′)–O(19) measures

Table 1. Bond distances for mamanine (**4**) in angstroms. The number in parentheses is the estimated error in the least significant figure

N(1)	- C(2)	1.473(6)	N(1′)	- C(2′)	1.479(5)
N(1)	- C(6)	1.486(5)	N(1′)	- C(6′)	1.484(6)
N(1)	- C(10)	1.460(5)	N(1′)	- C(10′)	1.474(5)
C(2)	- C(3)	1.514(6)	C(2′)	- C(3′)	1.508(7)
C(3)	- C(4)	1.512(7)	C(3′)	- C(4′)	1.502(9)
C(4)	- C(5)	1.521(8)	C(4′)	- C(5′)	1.533(7)
C(5)	- C(6)	1.535(6)	C(5′)	- C(6′)	1.530(6)
C(6)	- C(7)	1.549(6)	C(6′)	- C(7′)	1.523(6)
C(7)	- C(8)	1.523(6)	C(7′)	- C(8′)	1.512(6)
C(7)	- C(17)	1.523(6)	C(7′)	- C(17′)	1.533(7)
C(8)	- C(9)	1.522(5)	C(8′)	- C(9′)	1.526(6)
C(9)	- C(10)	1.527(6)	C(9′)	- C(10′)	1.518(6)
C(9)	- C(11)	1.507(5)	C(9′)	- C(11′)	1.500(6)
C(11)	- C(12)	1.344(6)	C(11′)	- C(12′)	1.365(6)
C(11)	- N(16)	1.374(5)	C(11′)	- N(16′)	1.361(5)
C(12)	- C(13)	1.402(7)	C(12′)	- C(13′)	1.401(7)
C(13)	- C(14)	1.357(7)	C(13′)	- C(14′)	1.365(7)
C(14)	- C(15)	1.409(6)	C(14′)	- C(15′)	1.417(6)
C(15)	- N(16)	1.379(5)	C(15′)	- N(16′)	1.384(5)
C(15)	- O(19)	1.267(5)	C(15′)	- O(19′)	1.256(5)
C(17)	- O(18)	1.439(6)	C(17′)	- O(18′)	1.339(7)

Table 2. Bond angles for mamanine (**4**) in degrees. The number in parentheses is the estimated error in the least significant figure

C(2)-N(1)-C(6)	112.3(3)	C(2′)-N(1′)-C(6′)	111.6(3)
C(2)-N(1)-C(10)	108.9(3)	C(2′)-N(1′)-C(10′)	108.9(3)
C(6)-N(1)-C(10)	112.7(3)	C(6′)-N(1′)-C(10′)	110.8(3)
N(1)-C(2)-C(3)	111.3(4)	N(1′)-C(2′)-C(3′)	112.5(4)
C(2)-C(3)-C(4)	109.9(4)	C(2′)-C(3′)-C(4′)	111.1(5)
C(3)-C(4)-C(5)	110.7(4)	C(3′)-C(4′)-C(5′)	110.0(4)
C(4)-C(5)-C(6)	113.4(4)	C(4′)-C(5′)-C(6′)	112.5(4)
N(1)-C(6)-C(5)	108.6(3)	N(1′)-C(6′)-C(5′)	108.5(3)
N(1)-C(6)-C(7)	110.3(3)	N(1′)-C(5′)-C(7′)	109.1(3)
C(5)-C(6)-C(7)	111.8(3)	N(5′)-C(6′)-C(7′)	112.3(3)
C(6)-C(7)-C(8)	110.4(3)	C(6′)-C(7′)-C(8′)	110.4(3)
C(6)-C(7)-C(17)	111.6(3)	C(6′)-C(7′)-C(17′)	113.2(4)
C(8)-C(7)-C(17)	109.7(3)	C(8′)-C(7′)-C(17′)	111.0(4)
C(7)-C(8)-C(9)	110.1(3)	C(7′)-C(8′)-C(9′)	111.4(4)
C(8)-C(9)-C(10)	109.0(3)	C(8′)-C(9′)-C(10′)	108.6(4)
C(8)-C(9)-C(11)	114.3(3)	C(8′)-C(9′)-C(11′)	112.9(3)
C(10)-C(9)-C(11)	110.3(3)	C(10′)-C(9′)-C(11′)	110.9(3)
N(1)-C(10)-C(9)	110.8(3)	N(1′)-C(10′)-C(9′)	112.9(3)
C(9)-C(11)-C(12)	127.0(4)	C(9′)-C(11′)-C(12′)	123.6(4)
C(9)-C(11)-N(16)	114.6(3)	C(9′)-C(11′)-N(16′)	117.6(3)
C(12)-C(11)-N(16)	118.4(3)	C(12′)-C(11′)-N(16′)	118.9(4)
C(11)-C(12)-C(13)	118.9(4)	C(11′)-C(12′)-C(13′)	119.4(4)
C(12)-C(13)-C(14)	122.5(4)	C(12′)-C(13′)-C(14′)	121.1(4)
C(13)-C(14)-C(15)	120.1(4)	C(13′)-C(14′)-C(15′)	120.3(4)
C(14)-C(15)-N(16)	115.4(4)	C(14′)-C(15′)-N(16′)	115.8(4)
C(14)-C(15)-O(19)	126.6(3)	C(14′)-C(15′)-O(19′)	124.9(4)
N(16)-C(15)-O(19)	117.9(3)	N(16′)-C(15′)-O(19′)	119.3(3)
C(11)-N(16)-C(15)	124.7(3)	C(11′)-N(16′)-C(15′)	124.6(3)
C(7)-C(17)-O(18)	111.5(4)	C(7′)-C(17′)-O(18′)	112.8(4)

173·4°. The two H-bonds cause an essentially flat 8-membered ring involving O(19), C(15), N(16), H(16), O(19), C(15'), N(16') and H(16') with a maximum deviation from the least squares plane of 0·1 Å.

Pohakuline (**5**), $C_{15}H_{26}N_2O_2$, crystallized in the chiral monoclinic space group C_2 with $a = 17{\cdot}685(2)$ Å, $b = 8{\cdot}943(1)$ Å and $c = 11{\cdot}533(2)$ Å and $\beta = 55{\cdot}47°$. A calculated density of 1·18 g/cm³ indicated one molecule per asymmetric unit. The X-ray experiment was identical with the one involving mamanine (**4**). A total of 1095 reflections were measured and 1067 (97%) were considered observed. ($F_o^2 \geq 3\sigma(F_o^2)$). The structure was solved using a multi-solution tangent formula approach.[15] Initially a 10-atom fragment was found which would not give proper phases for the rest of the molecule. This fragment was eventually found to be misplaced by approx. 0·5 Å in the z axis. 3-Dimensional electron density syntheses gave the rest of the molecule after the proper 10-atom fragment was introduced. The conventional discrepancy index was lowered to 0·033 in full matrix least squares refinements using anisotropic temperature factors for non-H atoms and isotropic temperature factors for H atoms.[16] Figure 2 is a computer-generated drawing of pohakuline (**5**).[17] Tables 3 and 4 contain the bond distances and bond angles which are in accord with commonly accepted values.

The conformation of rings A and B and the relation of the two substituents attached to ring B is the same for pohakuline (**5**) as for mamanine (**4**). However, the quinolizidine ring in pohakuline (**5**) adopts a pseudo-equatorial conformation relative to the α-piperidone moiety. The crystal structure of pohakuline (**5**) has only one intermolecular H-bond with distances of O(18)–H(18) 0·76 Å; N(1)–H(18) 2·20 Å; N(1)–O(18) 2·95 Å and a value of 171·4° for the angle O(18)–H(18)–N(1).

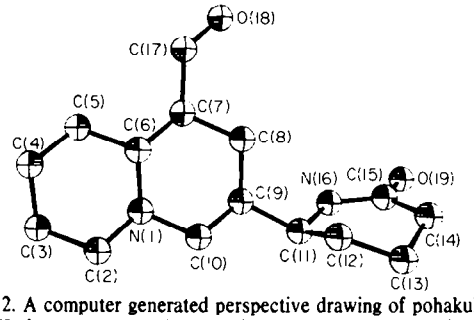

Fig. 2. A computer generated perspective drawing of pohakuline (**5**). Hydrogens are not shown and no absolute stereochemistry is implied.

Table 3. Bond distances for pohakuline (**5**) in angstroms. The number in parentheses is the estimated error in the least significant figure

N(1)	-	C(2)	1.477(3)
N(1)	-	C(6)	1.482(3)
N(1)	-	C(10)	1.467(2)
C(2)	-	C(3)	1.515(4)
C(3)	-	C(4)	1.512(4)
C(4)	-	C(5)	1.515(4)
C(5)	-	C(6)	1.528(3)
C(6)	-	C(7)	1.534(3)
C(7)	-	C(8)	1.527(3)
C(7)	-	C(17)	1.528(3)
C(8)	-	C(9)	1.515(3)
C(9)	-	C(10)	1.516(3)
C(9)	-	C(11)	1.541(3)
C(11)	-	C(12)	1.516(3)
C(11)	-	N(16)	1.467(3)
C(12)	-	C(13)	1.507(4)
C(13)	-	C(14)	1.504(4)
C(14)	-	C(15)	1.510(3)
C(15)	-	N(16)	1.335(3)
C(15)	-	O(19)	1.231(3)
C(17)	-	O(18)	1.412(3)

Table 4. Bond angles for pohakuline (**5**) in degrees. The number in parentheses is the estimated error in the least significant figure

C(2)	- N(1)	- C(6)	111.3(2)
C(2)	- N(1)	- C(10)	108.5(2)
C(6)	- N(1)	- C(10)	111.4(2)
N(1)	- C(2)	- C(3)	112.4(2)
C(2)	- C(3)	- C(4)	110.2(2)
C(3)	- C(4)	- C(5)	109.5(2)
C(4)	- C(5)	- C(6)	112.4(2)
N(1)	- C(6)	- C(5)	108.9(2)
N(1)	- C(6)	- C(7)	110.5(2)
C(5)	- C(6)	- C(7)	112.5(2)
C(6)	- C(7)	- C(8)	111.4(2)
C(6)	- C(7)	- C(17)	112.9(2)
C(8)	- C(7)	- C(17)	109.9(2)
C(7)	- C(8)	- C(9)	112.0(2)
C(8)	- C(9)	- C(10)	109.2(2)
C(8)	- C(9)	- C(11)	114.6(2)
C(10)	- C(9)	- C(11)	111.3(2)
N(1)	- C(10)	- C(9)	111.6(2)
C(9)	- C(11)	- C(12)	115.2(2)
C(9)	- C(11)	- N(16)	108.8(2)
C(12)	- C(11)	- N(16)	110.5(2)
C(11)	- C(12)	- C(13)	111.0(2)
C(12)	- C(13)	- C(14)	110.3(2)
C(13)	- C(14)	- C(15)	114.7(2)
C(14)	- C(15)	- N(16)	117.6(2)
C(14)	- C(15)	- O(19)	120.7(2)
N(16)	- C(15)	- O(19)	121.7(2)
C(11)	- N(16)	- C(15)	127.4(2)
C(7)	- C(17)	- O(18)	112.8(2)

EXPERIMENTAL

NMR spectra were obtained on Varian HA-100 and Varian XL-100 spectrometers using TMS as the internal standard. All spectra were recorded in the frequency sweep mode by Mr. James Loo. The signal multiplicities are denoted as singlet (s), doublet (d), triplet (t), and multiplet (m). Occasionally signals are also designated as being sharp (sp) or broad (bd).

All CMR spectra were recorded by Mr. James Loo on a Varian XL-100 spectrometer with TMS as internal standard. Chemical shifts are reported in δ units from TMS.

Mass spectra were recorded on a Hitachi Perkin-Elmer RMU-6D single focusing spectrometer at 20 and 70 eV by Sr. M. Roger Brennan.

IR spectra were recorded on a Beckman IR-10 spectrometer or a Perkin-Elmer 467 grating IR spectrometer. The spectra were obtained as chloroform solns with a cell path length of 0·1 cm, as KBr pellets, or as neat films. The absorption bands are reported in wavenumbers (cm^{-1}) and are designated as strong (S), medium (M), weak (W), shoulder (Sh), sharp (Sp) and broad (Bd).

UV spectra were taken in EtOH on a Beckman Acta C-III UV-visible spectrophotometer in 1 cm quartz cells.

Optical rotation was measured on an ETL-NPL Automatic Polarimeter Type 144A (Bendix Ericsson, U.K. Ltd.).

M.ps were taken on a Fisher-Johns apparatus and are uncorrected.

Silica gel HF-256 + 366 was used for all thin layer, basic or neutral alumina (Woelm) for column chromatography. Ascending paper chromatography on Whatman No. 1, BuOH/H_2O/HOAc (80:17:3).

Isolation and characterization. Collections at Pohakuloa, Hawaii, were made on 3-22-61, 5-20-61, and 7-14-61. Only the smooth young bark yielded alkaloids; extracts of the older, thick and more porous bark gave negative Dragendorff, Mayer and Wagner tests.

Dried and ground bark (1·78 kg) was extracted with MeOH for 93 hr, and the extract was evaporated to dryness *in vacuo*, leaving a dark brown gum. This gum was treated with 10% HCl until it was converted into a granular solid which was either centrifuged or filtered off. The solid was washed with 5% HCl until the washes no longer gave a positive Mayer's test. The solution was then thoroughly washed with $CHCl_3$, slowly made very alkaline with solid KOH (with cooling) and extracted with $CHCl_3$. Evaporation under vacuum of the $CHCl_3$ extracts, dried over Na_2SO_4, gave a crude alkaloid, 1·82 g (0·10% of dried bark). Further continuous extraction of the strongly basic aqueous soln with $CHCl_3$ gave a hygroscopic mixture (2·79 g) which showed a positive Mayer's test, and from which only a non-nitrogenous solid was isolated in quantity.

The initial crude alkaloid extract was triturated with small

portions of light petroleum until a very stiff oil was obtained, and this in turn was treated in the same manner with small portions of anhydrous ether, producing ultimately a buff powder. This powder was treated with a small amount of acetone which removed a dark red impurity, leaving a white solid (525 mg) on filtration, which was crystallized from acetone, giving a base which produced a single spot on paper chromatography, subsequently named pohakuline (**5**).

Similar treatment of the crude alkaloidal material from bark of the two later collections did not yield any acetone insoluble material. On chromatography over Woelm basic alumina I in benzene, the mixture of alkaloids was separated into matrine and cytisine.

Cytisine (**1**) was purified by sublimation (85–100°/>10 $^{-1}$ mm) of the material obtained from alumina chromatography. When recrystallized from acetone, the compound melted at 154°, gave a single spot on paper chromatography, R_f = 0·21, and a red color with aqueous $FeCl_3$ and had an IR spectrum identical with that of an authentic sample of cytisine (Mann Research Laboratories).

Matrine (**3**) was found predominantly in the light petroleum washes of the crude alkaloid mixture and was purified by chromatography over alumina followed by crystallization from light petroleum. This compound gave a single spot on paper chromatography, R_f = 0·40, and analyzed correctly for matrine. Its rotation in 95% EtOH and H_2O are +29·1° and +34·5° respectively. Mixture m.p. was undepressed and the IR spectrum was identical with a sample of matrine kindly supplied by Professor Y. Tsuda.

Pohakuline (**5**) gave no color with aqueous $FeCl_3$. It was crystallized from acetone, m.p. 170–171°, R_f = 0·36, $[\alpha]_D^{25}$ −17·2° (*c* 0·82 EtOH). (Found C, 67·49, 67·39; H, 9·73, 9·59; N, 10·09; $C_{15}H_{26}N_2O_2$ requires: C, 67·63; H, 9·84; N, 10·52%).

Treatment of a small amount of this base with $HAuCl_4$ gave an amorphous yellow solid which could not be crystallized satisfactorily, m.p. 160–165°.

LAH reduction. Pohakuline (215 mg, m.p. 171°) in anhydrous benzene was heated under reflux with a suspension of LAH (200 mg) for 24 hr. The cooled mixture was worked up with saturated Na_2SO_4 soln, and the product was obtained as a white solid (187 mg). This was crystallized from acetone, m.p. 155–156°, $[\alpha]_D^{28}$ +16·79° (*c* 2·4 EtOH). (Found: C, 71·96, 71·74; H, 11·36, 11·08; $C_{15}H_{28}N_2O_2$ requires: C, 71·38; H, 11·18%).

A fourth collection was made at Pohakuloa, Hawaii, on 2-22-1963. Ground bark (1·6 kg) was extracted with MeOH for 56 hr and MeOH was removed under diminished pressure. The remaining brown resinous matter was treated with 10% HCl (600 ml) until it was converted into a suspension of fine particles. It was filtered with the aid of diatomaceous earth. The remaining solid was digested again with 5% HCl acid (200 ml) and filtered. The treatment was repeated 4 times. The combined filtrate was washed with chloroform (3 × 200 ml), made basic with pellets of KOH (pH = 11–12) and extracted with chloroform (12 × 200 ml). The basic soln was concentrated *in vacuo* to half its volume and extracted again with chloroform (6 × 200 ml). The combined extract was dried with Na_2SO_4 and evaporated *in vacuo* to give a sticky oil (16·06 g).

A chloroform soln (25 ml) of the oil was applied to an alumina column (Woelm, neutral, activity II–III—4·5 ml of water every 100 g of the alumina—800 g) and successively eluted with chloroform (1·4 l), 2% MeOH (2 l) and 5% MeOH in chloroform (4 l).

Fraction 1 was a brown oil with the same R_f-value as that of authentic matrine on thin layer chromatography (alumina, benzene; MeOH 95:5). Fraction 3 was recrystallized from acetone to give colorless needles (0·50%), m.p. 154–155°, identical in all respects with cytisine.

On trituration of fraction 5 with acetone, there were obtained colorless rhombic prisms, 54 mg (0·0034%), m.p. 170–171°, identical with a sample of pohakuline.

On recrystallization from aqueous acetone (<1% water by volume) fraction 6 gave colorless crystals of **4**, 1·45 g (0·09%), m.p. ~100°, solidifying again at 120–140°, finally melting at 171–172°, $[\alpha]_D^{24.1}$ +31·7° (*c* 2·32 EtOH). (Found: C, 61·65, 61·64; H, 8·96, 8·93; N, 9·13; O, 20·51. $C_{15}H_{22}N_2O_2 \cdot 2H_2O$ requires: C, 60·39; H, 8·78; N, 9·39; O, 21·44%). When mamanine (100 mg) and cyclohexylsulfamic acid (400 mg) were dissolved in hot acetone (20 ml) and the soln was concentrated to 5 ml and kept at room temp for 4 d, a cyclohexylsulfamate separated as colorless crystals, m.p. 245–252°.

Mamanine (**4**). IR ($CHCl_3$): 3650 (W), 3490 (M, Bd), 3140 (W), 2780–3010 (S, complex shape stretching), 1660 (S, Sp), 1620 (S, Sp), 1560 (M, Sp) and 1465 (M, Sp) cm^{-1}. UV (MeOH): 233 (3·86), 308 (3·84) nm; (MeOH, H$^+$): 219, 289 nm. Ms (70 eV): *m/e* 262 (79), 245 (15), 231 (35), 204 (100), 127 (26), 124 (18), 122 (66), 121 (83), 84 (77), 83 (70 rel. %). NMR (DMSO-d_6): δ 1·0–1·95 (complex m, 8 H), 1·95–2·27 (m, 4 H), 2·74 (bdm, 4 H), 2·81 (bdm, 1 H), 3·40 (large, incl. H_2O), 4·42 (bds, 1 H), 6·00 (dd, 1 H, J = 1·7 Hz), 6·15 (dd, 1 H, J = 1·9 Hz), 7·35 (dd, 1 H, J = 7·9 Hz), 11·53 (bd, ~0·5 H); on D_2O addition signals at 4·42 and 11·53 collapse and the large peak at δ 3·40 collapses to a 2-H bd singlet. CMR (DMSO-d_6): 163·3 (sC-2), 151·8 (sC-6), 141·1 (dC-4). 116·8 (dC-3), 101·8 (dC-5), 62·9 (dC-10), 62·2 (tC-17), 60·1 (tC-16), 55·9 (tC-14), 43·3 (dC-9), 38·9 (dC-7), 33·5 (tC-11), 29·2 (tC-8), 25·3 (tC-13), 24·2 (tC-12).

Mamanine diacetate (**9**). Mamanine (~60 mg) was treated with Ac_2O in dry pyridine at room temp. for 1 day. To be assured of complete reaction, the mixture was chromatographed on a silica gel t.c plate using acetone as the developer. Two spots were observed—pyridine at R_f = 0·42 and the reaction product at R_f = 0·17. Excess pyridine and Ac_2O were removed *in vacuo*. A clear yellow oil showed one spot at R_f = 0·17. Upon standing for more than a day, the diacetate decomposes; it darkens in color and strongly smells of AcOH. NMR ($CDCl_3$): δ 1·30–2·18 (complex m, 8 H), 2·18–2·65 (m, 4 H), 2·07 (s, due to acetone), 2·09 (s, 3 H), 2·38 (s, 3 H), 3·06 (m, 1 H), 3·17 (m, 1 H), 3·28 (m, 1 H), 4·15 (s, 2 H), 6·99 (d, 1 H, J = 9 Hz), 7·18 (d, 1 H, J = 7 Hz), 7·76 (dd, 1 H, J = 7, 9 Hz). Ms (70 eV): *m/e* 346 (20), 303 (26), 287 (81), 246 (100), 245 (36), 243 (34), 204 (26), 164 (29), 122 (73), 121 (52), 110 (31), 97 (27), 96 (65), 84 (44), 83 (57), 55 (23), 43 (48 rel.%).

Pohakuline (**5**). IR ($CHCl_3$): 3640 (W), 3395 (M, Bd), 2770–2985 (S, complex shape stretching), and 1650 (S, Sp) cm^{-1}. Ms (70 eV): *m/e* 266 (48), 235 (13), 180 (18), 169 (34), 168 (56), 126 (33), 110 (24), 98 (60), 97 (100), 84 (71), 83 (51), 55 (53 rel.%). NMR ($CDCl_3$): δ 0·95–2·15 (complex m, 17 H), 2·26 (m, 2 H), 2·74 (s, 1 H), 2·82 (m, 1 H), 3·22 (m, 1 H), 3·59 (dd, 2 H, J = 1, 2·5 Hz), 5·94 (s, 2 H). D_2O exchange caused the lowest field signal at δ 5·94 to collapse.

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