SiO_2 with a 3:1 mixture of hexane:chloroform. The tetroxanes **6a** and **6b** (63 mg, 2.1%) were obtained as a mixture. Further chromatography of this mixture with hexane gave nearly complete separation of the two isomers.

Thermal Decomposition of 6a and 6b. A solution of **6a** and **6b** in $CDCl_3$ was sealed in a ^1H NMR tube and held at 20 °C, with examination by NMR at intervals. There was a steady decrease in the intensity of the resonance for **6b** (δ 2.11) and a concomitant increase in that of **7** (δ 2.18). A half-life of ca. 60 h at 20 °C could be calculated for **6b**. After **6b** was almost gone, the sample was held at 47 °C till only **6a** and **7** remained (ca. 5 h). The decomposition of **6a** was followed at 65 °C by observing the decrease in intensity of its resonance at δ 2.05. A half-life of ca. 4 h at 65 °C was calculated for **6a**. Admixture of authentic diacetyl peroxide gave only an increase in the intensity of the resonance at δ 2.18. Extraction of the $CDCl_3$ solution with D_2O had a negligible effect on the intensity of this resonance.

Caution! Compounds **6a** and **8** are reported to have exploded when exposed to heat or shock.[1] We experienced no explosions with **5**, but on several occasions, either neat or in concentrated solutions, it decomposed rapidly on being warmed to room temperature, with evolution of a gas and considerable heat. Great caution in working with these substances is urged.

Acknowledgment. We thank Dr. B. Wrackmeyer, Department of Inorganic Chemistry, Munich University, for a 50-MHz ^{13}C spectrum of **6b**.

Registry No. **1**, 1587-29-7; **5**, 90584-32-0; **6a**, 90584-33-1; **6b**, 90584-34-2; **8**, 90584-35-3; **10**, 90584-36-4.

Funebrine, a Structurally Novel Pyrrole Alkaloid, and Other γ-Hydroxyisoleucine-Related Metabolites of *Quararibea funebris* (Llave) Vischer (Bombacaceae)

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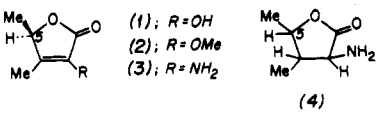
The flowers of the Mexican tree *Quararibea funebris* (Llave) Vischer (Bombacaceae) have been shown to give rise to the enolic γ-lactone **1**, its amino analogue **3**, the saturated lactone **4**, and the parent amino acid (2*S*,3*S*,4*R*)-γ-hydroxyisoleucine (**5**), as well as the novel pyrrole alkaloid funebrine (**6**). The structure determination of funebrine by X-ray crystallography is discussed and a hypothesis for its biogenesis offered.

The plant family Bombacaceae comprises 31 genera and 225 species distributed among six tribes.[2] Despite the economic significance of *Ceiba* and *Ochroma* species as sources of kapok and balsa wood, respectively, the secondary metabolites of members of the family have been little investigated. Our attention was drawn to the genus *Quararibea* partly by its equivocal taxonomic position[3] but principally because of its ethnobotanical interest.

Early Spanish explorers observed the Zapotec Indians near Oaxaca, Mexico, conducting funerary rites beneath the branches of the tree *Q. funebris*. The strongly odorous flowers of the tree have also been used since pre-Columbian times as an additive to chocolate drinks, known locally as *ponzonque* or *tejate*. In local folk medicine the plant is known as *flor de cocoa* and is used to control psychopathic fears, to regulate the menses, and as a cough remedy.[4] We also noted a previous report[5] suggesting the presence of alkaloids in the flowers.

The dried, milled flowers were extracted with hexane and then with 95% ethanol. The hexane extract yielded waxy hydrocarbons, fatty acid esters of C_{29} and C_{30} triterpenoids, and related compounds which will be reported elsewhere.

The 95% ethanol extract was partitioned into alkaloidal, phenolic, neutral chloroform-soluble, and water-soluble fractions. The phenolic fraction, in which the odor was concentrated, consisted of one major and many trace compounds. Flash chromatographic purification yielded the major component, a volatile, odorous compound of molecular formula $C_6H_8O_3$. A positive ferric chloride test, an O–H stretching band (3300 cm^{-1}) in the infrared spectrum, and other bands at 1750 and 1670 cm^{-1} [6] suggested an enolic lactone. The ^1H and ^{13}C NMR data, taken together with the ultraviolet spectrum (λ_{max} 232 nm, ϵ 7250, bathochromic shift of 42 nm in the presence of NaOH) are best accommodated by structure **1**. The compound is optically active ($[\alpha]_D^{25}$ −4.7°) and the absolute configuration shown in structure **1** (5*R*) is preferred on the basis of data given below. Compound **1** has not previously been obtained from a plant or animal source, but the racemate is known as the major flavoring constituent of aged sake.[7] Treatment of **1** with diazomethane yielded the methyl ether **2**.



Flash column chromatography of the basic fraction yielded two ninhydrin positive liquid compounds. The more abundant was assigned molecular formula $C_6H_9NO_2$ and structure **3**. In the electron-impact mass spectrum, fragments at m/z 99 (M − CO) and m/z 82 (M − COOH) indicate a lactone,[8] and the infrared carbonyl absorption

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(2) Hutchinson, J. "The Genera of Flowering Plants"; 1967; Vol. II, pp 522–531.

(3) Vischer, W. *Bull. Soc. Bot. Geneve* **1919**, II. Garcia-Barriga, H. *Mutisia* **1959**, *2*, 1. Alverson, B., personal communication, 1982. Schultes, R. E. *Bot. Mus. Leafl.*, Harv. Univ. **1957**, *17*, 247.

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(5) Rosengarten, F. *Bot. Mus. Leafl.*, Harv. Univ. **1977**, *25*, 184.

(6) Silverstein, R. M.; Bassler, C. G. "Spectrometric Identification of Organic Compounds," 2nd ed.; New York, 1968; p 92.

(7) Takahashi, K. *Agric. Biol. Chem.* **1976**, *40*, 325. Sulsur, H. *Z. Lebensm. Unters.-Forsch* **1972**, *148*, 215.

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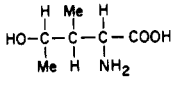
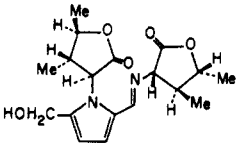
at 1750 cm^{-1} is in accord with an α,β-unsaturated γ-lactone. The ^1H and ^{13}C NMR spectra of the compound are in full accord with structure **3**. This is to our knowledge a new compound. The 5*R* configuration is also assigned to this compound on the basis of data given below. The minor compound, $C_6H_{11}NO_2$, had infrared carbonyl absorptions characteristic of primary amine (3385, 3315 cm^{-1}) and saturated γ-lactone (1775 cm^{-1}) groups. The ultraviolet spectrum (λ_{max} 208 nm) indicated no conjugation. In the ^1H NMR spectrum there are signals for two methyl groups each split by one proton (δ 1.20, J = 6 Hz; 1.45, J = 6 Hz). A 1 H doublet (δ 3.40, J = 11.8 Hz) can be assigned to a proton on carbon bearing nitrogen. There is also a 1 H doublet of quartets (J = 11 Hz, 6 Hz) at δ 4.25, arising from a proton on carbon bearing alkyl oxygen.

The electron-impact mass spectrum shows major peaks at M − CO_2 and M − CO_2 − CH_3, characteristic of methyl-substituted γ-lactones.[8] The data are best accommodated by structure **4**, a saturated derivative of structure **3**. The absolute configuration of compound **4** was established from the positive Cotton effect in circular dichroism (CD),[9,10] which is characteristic of α-amino lactones formed from L-α-amino acids. Taken together with this information, the J values for the methine protons allow the absolute stereochemical designation of the lactone to be established as 3*S*,4*S*,5*R*. This is to our knowledge the first report of this compound as a natural product, although we note that the 3*S*,4*R*,5*R* diastereomer has been isolated as a hydrolysis product from γ-amanitin.[11]

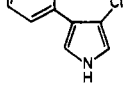
Support for the assigned configuration for this amino lactone came from the isolation of the corresponding amino acid from the water-soluble fraction of the extractives. This compound, mp 205–207 °C, $[\alpha]_D^{27.5}$ +2.90°, gave rise to a positive CD Cotton effect, in accord with an L configuration, and also, on heating in 1 N aqueous HCl, gave the amino lactone **4** in 87% yield. Structure **5**, (2*S*,3*S*,4*R*)-γ-hydroxyisoleucine, is therefore assigned to this apparently new compound. The diastereomer (2*S*,3*R*,4*R*)-γ-hydroxyisoleucine has been isolated from fenugreek seeds[11] and is also a component of γ-amanitin.[11]



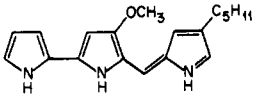
(5)



(6)



Pyrrolnitrin (7)



Prodigiosin (8)

The chloroform-soluble portion of the freeze-dried aqueous fraction gave weakly positive Dragendorff and Mayer tests for alkaloids, and a positive ferric chloride test. It was subjected to gradient elution flash column chromatography with silica gel as adsorbent. From two early fractions a crystalline substance was obtained having mp 230–233 °C. It gave positive Dragendorff and Mayer tests for alkaloids and a negative test with $FeCl_3$ and was named funebrine. The UV absorption spectrum [λ_{max} 293 nm (ϵ = 30 000), 265 nm (ϵ = 22 000)] was suggestive of an aromatic system. In the IR spectrum bands at 3430 and 1045 cm^{-1} indicated a primary alcohol, and a band at 1760 cm^{-1} a γ-lactone. Absorptions at 1650 and 1475 cm^{-1} suggested unsaturation, and several sharp intense bands in the region 1250 to 1050 cm^{-1} suggested an ester or lactone. The high-resolution mass spectrum gave a molecular ion (also the base peak) at 348.16890, corresponding to $C_{18}H_{24}N_2O_5$. This is suggestive of a very stable aromatic system. A peak at m/z 330 (M − H_2O) is consonant with an alcohol function, and a peak at m/z 315 signifies the loss of a methyl group and water. Besides the base peak, the most abundant ion in the mass spectrum was m/z 235 (intensity of 95%). Such a stable fragment denotes the presence of an aromatic system which is retained even after the loss of $C_6H_9O_2$ and suggested the presence of a γ-lactone similar to lactone **4** in the molecule. The peak at m/z 271 (M − (H_2O + CH_3 + CO_2)) represents a series of cleavages which is analogous to that shown by the amino lactone **4**.

In the ^1H NMR spectrum three doublets resonating at δ 1.05, 1.35, and 1.45 were assigned to three different methyl groups each split by a single proton, analogous to signals seen in the spectrum of the amino lactone **4**. A multiplet at δ 4.1 is also seen in the spectrum of funebrine and of the lactone **4**. It arises from a single proton attached to a carbon bearing oxygen. One proton was exchangable with D_2O. Two doublets at δ 6.70 and 6.30 (J = 3 Hz) are assigned to aromatic protons adjacent to each other.

X-ray crystal structure analysis was undertaken for the complete structure elucidation. Crystals initially obtained from chloroform–cyclohexane proved unsuitable, as did those grown from pyridine. Slow evaporation at room temperature of a solution in Me_2SO produced acceptable crystals. Structure **6** for funebrine resulted from the X-ray determination.[12,13] The molecular conformation of the molecule is depicted in Figure 1. The stereochemistry chosen is based on the configuration of the congeneric amino lactone **4**. Table I gives the positional parameters,

(8) Pfaender, P.; Wieland, T. *Leibigs Ann. Chem.* **1966**, *700*, 126.
(9) Snatzke, G. In "Methodum Chimicum"; F. Korte, Ed.; 1974; Vol. II, pp 428.
(10) Fowden, L.; Pratt, H. M.; Smith, A. *Phytochemistry* **1973**, *12*, 1707.
(11) Wieland, T.; Hasan, M.; Pfaender, P. *Liebigs Ann. Chem.* **1968**, *717*, 205.

(12) The structure elucidation of **6** was carried out by single-crystal X-ray analysis. Preliminary photographs showed monoclinic symmetry. Accurate cell dimensions, determined by a least-squares fit of 15 diffractometer-measured, high-order reflections, were a = 7.492 (4) Å, b = 8.052 (4) Å, c = 15.278 (2) Å and β = 91.98 (1)°. Systematic absences ($0k0$ $\neq 2n$) and the optical activity fixed the space group as $P2_1$; a density of 1.25 g cm^{-3} indicated one molecule per asymmetric unit. A quadrant of data to 2θ = 115° was collected by the θ–2θ scanning technique on a Syntex $P2_1$ automated diffractometer by using Cu K radiation (λ = 1.5418 Å). After Lorentz, polarization and absorption corrections were made, 1127 reflections were considered observed [$|F_o| \geq 3.0\sigma|F_o|$] and were used in the analysis. Initial attempts to solve the structure by using the direct phasing methods in MULTAN did not yield a reasonable phasing model. The MAGEX phase determining procedure, used in concert with MULTAN, yielded a thirteen atom fragment, which was ultimately developed into the entire molecule. Originally all nonhydrogen atoms were refined as carbon atoms and temperature factors were monitored to identify nitrogen and oxygen atoms. Bond lengths and temperature factor behavior allowed unequivocal assignment of structure. Full-matrix least-squares refinement (anisotropic for non-hydrogen atoms, isotropic for hydrogen atoms) led to a residual of 0.057.[13]

(13) All crystallographic calculations were carried out on a VAX 11/780 computer. The principal programs used were: FMLS, anisotropic full-matrix least-squares refinement (Ganzel, P. L.; Sparks, R. A.; Trueblood, K. N. UCLA; modified by McPhail, A. T., Duke University), ORTEP, crystallographic illustration programs (Johnson, C. K., Oak Ridge), ORNL-3974, MULTAN 80 (Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. B* **1970**, *B26*, 274), MAGEX, a phase determination procedure (Hull, S. E.; Viterbo, D.; Woolfson, M. M.; Shao-Hui, Z., University of York).

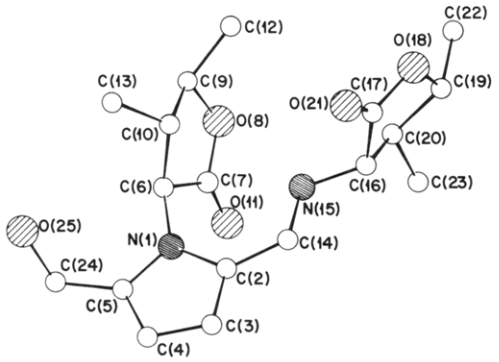


Figure 1. Molecular conformation and numbering scheme for funebrine. Oxygen and nitrogen atoms have been crosshatched and hydrogen atoms have been omitted.

Table II shows the bond lengths and valency angles, and Table III gives the torsion angles for the non-hydrogen atoms in **6** (see supplementary material).

The bond lengths and angles manifest the extent of delocalization through the molecule. The exocyclic C-(14)–N(15) imine bond lies syn planar to the N(1)–C(2) bond (N(1)–C(2)–C(14)–N(15) 1.1°) as expected for optimized interaction with the aromatic π system. The C-(24)–O(25) length (1.400 (7) Å) is rather short but a similar distance (1.398 Å) was found in β-(hydroxymethyl)-pyrrole.[14]

The configurations of the two amino lactone fragments are the same and analogous envelope conformations are adopted; C(10) lies 0.46 Å from the plane containing C(6), C(7), O(8), and C(9), and C(20) lies 0.54 Å from the plane containing C(16), C(17), O(18), and C(19). The methyl groups on the lactone moieties appear quite anisotropic which may explain the rather short C–C bond lengths (range 1.501–1.514 Å).

Molecules are held together in the x direction by hydrogen bonds between an O(25) hydroxyl group and an O(11) carbonyl oxygen atom of a molecule related by −1 + x, y, z [O(25)···O(11) 2.81 Å].

The high extinction coefficient in the UV spectrum of funebrine is due to the conjugation of the imino group with the pyrrole ring. In the ^{1}H NMR spectrum a signal at δ 8.19 is assigned to the imine proton. The two doublets with large J values (J = 10.5 Hz) at δ 5.70 and 3.90, which integrate for a single proton each, are due to the protons on C-6 and C-16 which are trans to the C-10 and C-20 protons, respectively. The assignment of the C-6 proton further downfield than the proton at C-16 is due to the deshielding effect of the neighboring aromatic group on the C-6 proton. The two pyrrole protons are seen at δ 6.70 and 6.30. The benzylic protons on C-24 and the alcohol proton all occur together at δ 4.90. The major cleavage in the mass spectrum is the loss of the $C_6H_9O_2$ fragment to produce m/z 235. This can now be ascribed to the loss of one of the lactones. The facile cleavage between N-1 and C-6 is suggested because it produces the most stable cation.

We suggest that funebrine arises biogenetically by the condensation of a derivative of a six-carbon sugar and two molecules of lactone **4**.

Funebrine is an entirely new alkaloid and its discovery represents the first characterization of an alkaloid in the family Bombacaceae; it appears also to represent a novel alkaloidal structural type. The pyrrole alkaloids are nowhere common. Interestingly, they have been found in certain species of bacteria, namely, *Pseudomonas pyrocinia* and *Serratia marcescens*. Pyrrolnitrin **7** and prodigiosin **8** are examples of some pyrrole alkaloids.

(14) Sheldrick, W. S.; Borkenstein, A.; Engel, J. *Acta Crystallogr., Sect. B* **1978**, *B34*, 1248.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were determined on Perkin-Elmer Model 599B and 700 spectrophotometers. Unless otherwise stated, proton NMR spectra were obtained on a Varian T-60 spectrometer. ^{13}C NMR spectra were determined on a JOEL FX-60 Q (pulsed Fourier transform) instrument also with $CDCl_3$ as solvent, and chemical shifts are given as ppm downfield from Me_4Si as an internal standard. Ultraviolet (UV) spectra were obtained on a Beckman DB-G spectrophotometer. Optical rotations were determined on Perkin-Elmer 241 polarimeter with a 1-dm cell by using sodium and mercury lamps as sources. Circular dichroism (CD) spectra were obtained on a Cary 60 spectropolarimeter with a Model 6001 CD attachment. HPLC was run on a Waters 6000 pump with a Spectromonitor III variable UV wavelength detector. HPLC columns were either Waters 10-μm C^{18} or 10-μm Partisil silica gel.

Low-resolution mass spectra were obtained with a Nuclide 12-90-G spectrometer and a Finnigan 4021 quadrupole spectrometer; high-resolution mass spectra were made on a CEC 21-110B instrument. Unless otherwise noted, standard deviations are ±0.00005 amu. Chemical-ionization (CI) mass spectra were obtained by using NH_3 as the ionizing gas on a Finnigan MAT 311. Field-desorption (FD) mass spectra were determined on a Varian (Finnigan) MAT 731.

Column chromatography was performed with alumina or silica gel, E. Merck 7734, 70–230 mesh (63–200 μm particle size). Flash column chromatography (FCC) utilized silica gel 60, E. Merck 9385, 230–400 mesh (40–63 μm particle size) in a 50-mm width glass column. UV light was used to monitor elution of fluorescent material on both types of column chromatography. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 pre-coated plates (200 μm plate thickness), Baker (7001) or E. Merck (5539 and 5760), and precoated microcrystalline cellulose, Baker (5080).

Preparative TLC utilized 2 mm thick silica gel 60 plates (20 cm × 20 cm, E. Merck 5766). All TLC plates, unless stated otherwise, contained a fluorescent indicator (F-254).

General purpose visualization of TLC plates was effected with UV light (short- and longwave) and iodine vapor. Specific types of compounds were visualized as follows: primary and secondary amines with ninhydrin, 0.3% in 97 mL of *n*-butanol and 3 mL of acetic acid (heated for 5 min at 100 °C); phenols and enols, 5% $FeCl_3$ in 0.5 N hydrochloric acid; alkaloids, Dragendorff and iodoplatinate spray reagents; sugars, spray of 1% aniline hydrogen phthalate in *n*-BuOH with heating (100 °C for 2 min).

Extractions and Partitions. Ground air-dried flowers (2.9 Kg) were extracted at room temperature with hexane (4.5 gal) 4 times (fresh, recovered hexane each time). The hexane extracts (fraction 1) were concentrated under vacuum to give a thick syrup (46.9 g). This gave moderate Dragendorff and Mayer tests. The hexane extract was then extracted with 5% HCl (four times) to remove basic material. This acid extract was made alkaline (pH 12) and extracted with ether. The ether extract was concentrated to yield 198 mg of a brown liquid. The marc, after air drying, was extracted to exhaustion with 5 gal of 95% ethanol (four times) and this extract was concentrated under vacuum to a thick syrup. This concentrate was partitioned between H_2O/MeOH (9:1) and $CHCl_3$ giving materials of fraction 2 and fraction 3, respectively.

Fraction 2 was concentrated under vacuum to remove the MeOH and subsequently freeze-dried to yield 303 grams of a yellow powder. The $CHCl_3$ (fraction 3) was extracted (four times) with 5% NaOH. The 5% NaOH extract was made acidic (pH 2) and extracted four times with ether to yield 4.08 grams of a pleasant smelling liquid (fraction 4), which gave a positive $FeCl_3$ test (purple). The remaining $CHCl_3$ (now fraction 5) was next extracted four times with 5% HCl. This acid extract was made basic and extracted four times with ether to yield 500 mg of brown liquid (fraction 6) which gave moderate Dragendorff and Mayer tests. The remaining $CHCl_3$ extract was washed twice with H_2O,

dried over sodium sulfate, and concentrated under vacuum to give 37.5 grams of syrup (fraction 7).

Fraction 2 (freeze-fried aqueous extract) was reconstituted 20% weight/volume with 5% NaOH and extracted (four times) with $CHCl_3$ to remove basic compounds; yield, 1.00 g. The remaining basic extract was then made neutral (pH 7) and extracted with $CHCl_3$ (four times) (fraction 8) and EtOAc (four times) (fraction 9). These two extracts were concentrated to yield 350 mg and 1.5 g, respectively, of materials.

Constituents of the Combined Alkaloidal Fractions. The basic and alkaloidal compounds removed from fractions 1 and 2 by extraction and fraction 6 were combined because of similar TLC profiles, using silica gel with 5% methanol in $CHCl_3$ as eluant and spraying with Dragendorff's reagent and ninhydrin. The combined weight was 1.798 g (0.0625%) of basic material.

(1) Chromatography. The combined basic extracts (1 g) was subjected to gradient elution FCC on a 5 cm × 200 cm column with 100 grams of 230–400 mesh silica gel. Thirty-four fractions were collected. All fractions were monitored by using TLC on silica gel 60 with 2% methanol in chloroform as eluant and combinations were made on the basis of similarity. Fractions 1–9 (methanol/chloroform 2:98) comprised 231 mg, fractions 10–14 (methanol/chloroform 1:9) 113 mg, fractions 19–24 (methanol/chloroform 1:4) 132 mg, and fractions 27–31 (methanol) 194 mg. Fractions 15–31 all contained alkaloidal material.

The ninhydrin-positive fractions were combined and rechromatographed with FCC with 1% methanol in chloroform as eluant. Fifteen fractions were collected. Fractions 2–5 (150 mg) contained a single noncrystallizable liquid substance and fractions 7–10 (45 mg) contained a second, single, noncrystallizable compound.

(2) Characterization of Ninhydrin Positive Material. Fractions 2–5 (150 mg) proved to be 3-amino-4,5(*R*)-dimethyl-2(5*H*)-furanone (3): R_f 0.75 with silica gel 60 and 5% methanol in chloroform as eluant and R_f 0.41 with ether; UV λ_{max} 257 nm, 206 nm shoulder (ϵ 11 700, MeOH); IR (neat on NaCl plates) ν_{max} 3460, 3370, 3230, 2980, 2945, 2875, 1750, 1700, 1615, 1340, 1225, 1150, 1050, 925, 650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (d, J = 6 Hz) CH_3, 1.8 (d, J = 1 Hz) CH_3, 3.45 (s) NH_2, exchanges with D_2O, 4.75 (q, J = 7 Hz) OCH; ^{13}C NMR ($CDCl_3$) δ 9.8 (q, J = 128.3 Hz) CH_3, 18.6 (q, J = 129.0 Hz) CH_3, 78.6 (dd, J = 150.2 and 4.4 Hz) OCH, 128.1 (s) CH_3C=, 129.0 (s) NH_2C=, 170.8 (s) C=O; low-resolution MS 128 (10%) M + 1, 127 (80%) M^+, 112 (30%) M - CH_3, 99 (45%) M - CO, 84 (30%) M - CH_3 - CO, 82 (30%) M - CO_2H, 72 (100%) M - 55, 56 (80%), 43 (100%), 27 (60%); optical rotations $[\alpha]^{24}_D$ -3.0° (c 0.53, MeOH), with a mercury lamp $[\alpha]_{578}$ -3.2°, $[\alpha]_{546}$ -4.5°, $[\alpha]_{436}$ -10.0°, $[\alpha]_{365}$ -9.2°.

Fractions 7–10. (45 mg) were identified as 3(*S*)-amino-4-(*S*),5(*R*)-dimethyl-2-oxo-tetrahydrofuran: TLC (free base) R_f 0.41 with 8% methanol in chloroform on silica gel 60.

The HCl salt was prepared as follows. The amino lactone dissolved in ether was added dropwise to HCl saturated ether with stirring. The white powder was filtered and dried over boiling toluene in vacuo: mp 212–215 °C; UV (for HCl salt) λ_{max} 208 nm (ϵ 339, MeOH); IR (free base neat on NaCl plates) ν_{max} 3520, 3385, 3315, 3200, 2980, 2935, 2880, 1775, 1600, 1190, 1175, 1145, 1040, 910, 700 cm^{-1}; ^1H NMR ($CDCl_3$) free base δ 1.2 (d, J = 6 Hz) CH_3, 1.45 (d, J = 5.5 Hz) CH_3, 1.75 (s) NH_2, exchanges with D_2O, 2.0 (m) CH, 3.4 (d, J = 11.8 Hz) NH_2CH, 4.25 (d, q, J = 10.5 and 6.5 Hz) OCH; ^{13}C NMR ($CDCl_3$) free base 13.0 (q, J = 127 Hz, d, 4.0 Hz) CH_3, 17.6 (q, J = 127 Hz, d, 4.8 Hz) CH_3, 46.3 (d, J = 129 Hz, d, 3.5 Hz) $HCCH_3$, 57.8 (d, J = 132 Hz, d, 4.0 Hz) $HCNH_2$, 78.6 (d, J = 147 Hz, d, 4.8 Hz) HCO, 177.3 (d, J = 5.3 Hz) C=O; low-resolution MS 130 (2%) M + 1, 129 (10%) M^+, 100 (17%) M - 29, 85 (46%) M - CO_2, 70 (100%) M - CO_2CH_3, 57 (29%); optical rotation $[\alpha]^{25}_D$ -14.8° for HCl salt (c 0.695, MeOH), $[\alpha]_{578}$ -15.15°, $[\alpha]_{546}$ -16.5°, $[\alpha]_{446}$ -20.6°, $[\alpha]_{365}$ -12.7°.

A positive Cotton effect was observed in the circular dichroism spectrum: molar ellipticity $[\theta]$ = +2900 ($\Delta\Sigma$ +0.88) at 212.5 nm for a 10-mM solution of the HCl salt.

Constituents of the Phenolic Fraction 4. Fraction 4 (2.5 g) was separated into 31 fractions with FCC with silica gel and 2% methanol in chloroform as the initial eluant and doubling the amount of methanol in chloroform until pure methanol was the eluant. Fractions 2–5 (methanol/chloroform 2:98) contained the bulk of the material (1.5 g) and gave a positive $FeCl_3$ test (purple). TLC gave an R_f of 0.40 in 2% methanol in chloroform which was $FeCl_3$ positive and quenched F-254 silica gel plates under UV light. The material gave a positive tetranitromethane test (a few mg of tetranitromethane dissolved in 1 mL of chloroform is added to a $CHCl_3$ solution of the compound to be tested and the production of a yellow color is considered positive for the presence of unsaturation). Fractions were again chromatographed with FCC with silica gel and 0.5% methanol in chloroform. Of 23 fractions that were collected, fractions 11–14 contained a homogeneous liquid compound (TLC), with an odor of vanilla (505 mg).

This compound was identified as 3-hydroxy-4,5(*R*)-dimethyl-2(5*H*)-furanone (1): R_f 0.40 with 2% methanol in chloroform and 0.10 using 3:7 acetone/cyclohexane (quenches F-254 silica plates and turns purple with $FeCl_3$ spray reagent); UV λ_{max} 232 nm (ϵ 7250, MeOH) (bathochromic shift of 42 nm with the addition of 1 drop of 1.0 N NaOH); IR (neat on NaCl plates) ν_{max} 3300, 2990, 2920, 2860, 1755, 1700, 1670, 1515, 1252, 1178, 1050, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (d, J = 6 Hz) CH_3, 1.90 (s) CH_3, 4.90 (q, J = 7 Hz) HCO, 7.12 (s) OH (disappears with addition of D_2O); ^{13}C NMR ($CDCl_3$) δ 9.2 (q) CH_3, 18.4 (q) CH_3, 78.2 (d) OCH, 133.5 (s) COH, 137.8 (s) CH_3C=, 170.8 (s) C=O; field-desorption MS 151 (M + Na), 100 (M - CO); electron-impact low-resolution MS M+ 130 (4%) M + 2, 129 (26%) M + 1, 128 (38%) M^+, 113 (8%) M - CH_3, 100 (10%) M - CO, 85 (21%) M - CO - CH_3, 83 (100%) M - CO_2H, 72 (22%) M - 56, 57 (19%), 55 (33%), 43 (56%); high resolution MS M^+ 128.04839 ($C_6H_8O_3$), 113.02362 ($C_5H_5O_3$), 100.05182 ($C_5H_8O_2$), 85.02852 ($C_4H_5O_2$), 84.05463 (C_5H_8O), 83.05081 (C_5H_7O), 72.05748 (C_4H_8O), 71.04990 (C_4H_7O); optical rotation $[\alpha]^{25}_D$ -4.7° (c 0.85, CH_2Cl_2).

The Methyl Ether of Lactone 2. 2-Methoxy-4,5(*R*)-dimethyl-2(5*H*)-furanone (2) was synthesized from 250 mg of enol lactone 1 with ethereal diazomethane in the usual way. Workup involved evaporation of solvents in vacuo to yield 150 mg of the crude methyl ether, which was chromatographed by using preparative silica gel TLC plates and cyclohexane/ether (3:7). UV quenching material at R_f 0.40 was scraped off the plate and eluted with $CHCl_3$, yield 95 mg.

Analytical TLC gave R_f 0.45 with the same eluant as above. Spectral data: UV (cyclohexane) λ_{max} 227 nm, 201 nm (ϵ 6975, 2675); IR (neat on NaCl plates) ν_{max} 3500, 2990, 2940, 2835, 1765, 1695, 1450, 1340, 1235, 1145, 1070, 1060, 935 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (d, J = 6 Hz) CH_3, 1.95 (d, J = 1 Hz) CH_3, 3.95 (s) OCH_3, 4.8 (q, J = 6.5 Hz) HCO; low-resolution MS 143 (16%) M + 1, 142 (69%) M^+, 127 (16%) M - 15, 114 (75%) M - CO, 99 (100%) M - CO - CH_3, 71 (66%), 43 (38%); optical rotation $[\alpha]^{25}_D$ -16.8° (c 0.57, cyclohexane), with a mercury lamp $[\alpha]_{578}$ -17.7°, $[\alpha]_{546}$ -20.5°, $[\alpha]_{436}$ -38.9°, $[\alpha]_{365}$ -69.1°.

Constituents of the Aqueous Fraction 2. (1) Constituents of Fractions 8 and 9. The freeze-dried fraction (250 g) was reconstituted (20% weight/volume) with H_2O and extracted four times with $CHCl_3$ and four times with ethyl acetate. The $CHCl_3$ extract (fraction 8) and the EtOAc extract (fraction 9) yielded, after drying over Na_2SO_4 and concentration under vacuum, 1.3 g and 4.8 g, respectively.

Fraction 8 gave slight positive Dragendorff and Mayer tests and a purple-brown color with $FeCl_3$. The fraction was then chromatographed with gradient elution FCC on silica gel with cyclohexane/acetone (1:1) as the initial eluant. Twenty fractions of 50 mL each were collected. Fractions 5 and 6 yielded a white microcrystalline precipitate after crystallization from cyclohexane/$CHCl_3$ (1:1), mp 230–234 °C, weight 24 mg. Moderate Dragendorff and Mayer tests were observed; a green Ehrlich test was also observed. The crystals were appreciably soluble only in pyridine and dimethyl sulfoxide (Me_2SO). Slow evaporation from pyridine at room temperature gave thin clear plates, mp 231–233 °C, which proved unsuitable for X-ray studies. However, with Me_2SO and slow evaporation at room temperature (22 days), suitable rhomboid shaped crystals were produced, mp 232–233 °C. Single-crystal X-ray analysis were performed and the alkaloid identified as funebrine (6): IUPAC name, (*E*)-(3*S*,4*S*,5*R*)-Dihydro-3-[2-(hydroxymethyl)-5-[*N*-[(3*S*,4*S*,5*R*)-tetrahydro-4,5-dimethyl-2-oxo-3furyl]formimidoyl]pyrrol-1-yl]-4,5-dimethyl-2-(3*H*)-furanone; R_f 0.2 on silica gel G with ether as eluant, R_f 0.7 with ether/acetone (4:1); UV (Me_2SO) λ_{max} 293 nm (ϵ 30 000), sh 265 nm (ϵ 20 000); IR (KBr pellet) ν_{max} 3430, 3195, 2970, 2920, 2870, 1760, 1650, 1485, 1440, 1385, 1312, 1300, 1205, 1195, 1115,

1105, 1045, 1035, 975, 888, 790, 728, 692, 670, 595, 575, 500 cm^{-1}; ^1H NMR pyridine-d_5) δ 1.05 (d, J = 6 Hz) 6 H, CH_3 (23) and CH_3 (13), 1.35 (d, 6 Hz) 4 H, CH_3 (22) and C-H (20), 1.45 (d, 6 Hz) 4 H, CH_3 (12) and CH (10), 4.1 (m), 2 H, OCH (19) and OCH (9), 3.9 (d, 10.5 Hz), 1 H, HCN (16), 5.7 (d, 10.5 Hz) 1 H, HCN (6), 4.9 (s) 3 H, CH_2OH (exchanges with D_2O to integrate for 2 H), 6.3 (d, 3 Hz) 1 H (3), 6.7 (d, 3 Hz), 1 H (4), 8.19 (s) 1 H, HN=C; low-resolution MS 349 (28% M + 1, 348 (100%) M^+, 330 (59%) M - H_2O, 319 (60%) M - CHO, 315 (38%) M - H_2O - CH_3, 271 (25%) M - CO_2 - H_2O - CH_3, 235 (95%) M - lactone, 217 (42%) M - lactone - H_2O, 202 (18%) M - lactone - CH_3 - H_2O; high-resolution MS M^+ 348.16890 ($C_{18}H_{24}N_2O_5$), 330.16103 ($C_{18}H_{22}N_2O_4$), 319.16788 ($C_{17}H_{23}N_2O_4$), 315.13753 ($C_{17}H_{17}N_2O_4$), 286.17059 ($C_{17}H_{22}N_2O_2$), 271.14732 ($C_{16}H_{19}N_2O_2$), 261.12379 ($C_{14}H_{17}N_2O_3$), 236.11247 ($C_{11}{}^{13}C_1H_{15}N_2O_3$), 235.10820 ($C_{12}H_{15}N_2O_3$), 220.0948 ($C_{15}H_{12}N_2$), 217.09759 ($C_{12}H_{13}N_2O_2$), 202.08713 ($C_{12}H_{12}NO_2$), 191.12005 ($C_{11}H_{15}N_2O$), 163.08753 ($C_9H_{11}N_2O$), 159.09418 ($C_{10}H_{11}N_2$), 149.07322 ($C_8H_9N_2O$), 145.07680 ($C_9H_9N_2$), 136.06509 ($C_7H_8N_2O$), 119.06096 ($C_7H_7N_2$), 114.06848 ($C_6H_{10}O_2$), 113.06040 ($C_6H_9O_2$), 109.05272 ($C_6H_7NO$), 99.04468 ($C_5H_7O_2$), 92.04987 ($C_6H_6N$), 80.05201 ($C_5H_6N$); optical rotations $[\alpha]^{22.5}_D$ -215° (c 0.01, Me_2SO), $[\alpha]_{578}$ -225°, $[\alpha]_{546}$ -265°, $[\alpha]_{436}$ -436°, $[\alpha]_{365}$ -1125°.

Fractions 7, 8, and 9 yielded a small amount (100 mg) of enol lactone 1. A purple color was produced with 5% $FeCl_3$. Identification was confirmed by co-TLC and IR with the previously isolated enol.

(2) Isolation and Characterization of Amino Acid 5. The freeze-dried aqueous fraction (3 g) was reconstituted with water (50 mL) and extracted four times with water-saturated butan-1-ol. The aqueous fraction which was left after the butanol extraction (2.5 g) was concentrated and applied to a column containing 100 grams of cation (H^+) exchange resin (Zeolite AG 50 W-X2, 200–400 mesh). The column was eluted initially with 200 mL of H_2O to remove sugars. NH_4OH (100 mL, 0.5 N) was then added, followed by 1.0 N NH_4OH (200 mL). The NH_4OH eluants were collected and concentrated by freeze drying, yield 210 mg. Cellulose TLC with butanol/acetic acid/water (3:1:1) as eluant and ninhydrin as spray indicator revealed four spots with R_f of 0.66 (yellow), 0.51 (red), 0.44 (purple), and 0.38 (purple). The component at R_f 0.51 appeared to be the most abundant (approximately 80%) amino acid.

The crude amino acid mixture was separated by using isocratic FCC with 50 g of silica gel and chloroform/methanol/water (10:5:1) as eluant. Twenty fractions were collected. Fractions 12, 13, and 14 contained the crude amino acid as a white powder, yield 105 mg. This was then dissolved in hot 90% EtOH and allowed to crystallize at room temperature overnight; yield, 90 mg felted needles; mp 205–207 °C; R_f 0.5 on microcrystalline cellulose with butanol/acetic acid/water (3:1:1) as eluant (The spot turned red with ninhydrin and heat); UV (H_2O) λ_{max} 197 nm (ϵ 250); IR (KBr pellet) ν_{max} broad band from 3600–2000, 3305, 3130, 2975, 2920, 2560, 2010, 1635, 1595, 1525, 1405, 1355, 1145, 1065, 925, 530 cm^{-1}; ^1H NMR (D_2O) δ 0.95 (d, J = 6 Hz), CH_3, 1.25 (d, 5.5 Hz) CH_3, 2.15 (m) CH, 3.85 (m, 6 Hz), OCH, 4.0 (d, 2.2 Hz) $HCNH_2$; ^{13}C NMR (D_2O) δ 4.1 (q) CH_3, 23.2 (q) CH_3, 42.4 (d) CH_3CH, 58.2 (d) NH_2CH, 72.0 (d) OCH, 176.6 (s) C=O; low-resolution MS M^+ 129 (20%) M - H_2O, 103 (11%) M - CO_2, 102 (46%) M - CO_2H, 86 (12%), 85 (35%), 74 (29%), 71 (24%), 70 (100%), 58 (44%), 57 (37%), 56 (41%); field-desorption MS 148 (M + H); high-resolution MS M^+ 147.08745 ($C_6H_{13}NO_3$), 132.06607 ($C_5H_{10}NO_3$), 129.08095 ($C_6H_{11}NO_2$), 114.05597 ($C_5H_8NO_2$), 103.09524 ($C_5H_{12}NO$), 103.06333 ($C_4H_9NO_2$), 102.09425 ($C_4{}^{13}CH_{12}NO$), 102.05550 ($C_4H_8NO_2$), 88.03913 ($C_3H_6NO_2$), 87.04456 ($C_4H_7O_2$), 86.03678 ($C_4H_6O_2$), 86.06014 (C_4H_8NO), 86.09505 ($C_5H_{12}N$), 85.08978 ($C_5H_{11}N$), 84.08138 ($C_5H_{10}N$), 83.07357 (C_5H_9N), 75.03249 ($C_2H_5NO_2$), 74.02656 ($C_2H_4NO_2$), 70.06634 (C_4H_8N); optical rotations $[\alpha]^{27.5}_D$ +2.9° (c 0.104, H_2O), $[\theta]$ 6479, $\Delta\sum$ +1.96.

(3) Lactonization of Amino Acid 5. Compound 5 (50 mg) was heated with 5 mL of 1 N HCl in a sealed test tube at 100 °C for 20 min. The cooled solution was then made basic (pH 11) with 2 N NaOH and extracted three time with chloroform. The chloroform was dried over Na_2SO_4 and concentrated, yield 38.5 mg, 87.5% conversion. NMR and IR spectra were identical with those of the previously identified amino lactone 4. The lactone was converted to the HCl salt by adding it to HCl saturated ether, mp 212–214 °C; $[\alpha]^{25}_D$ -13.2° (c 0.2 in MeOH).

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Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, thermal parameters for the non-hydrogen atoms, hydrogen atom positional and isotropic thermal parameters, and bond lengths (5 pages). Ordering information is given on any current masthead page.