g, 49%): IR (neat, NaCl) 2924, 1695, 1678, 1570, 1462, 992 cm^{-1}; ^1H NMR (CCl_4) δ 7.37 (d, J = 19 Hz, 1 H) 6.38 (d, J = 19 Hz, 1 H) 2.47 (t, J = 6 Hz, 2 H) 1.90–0.70 (bm, 36 H). Anal. Calcd for $C_{20}H_{40}OSn$: C, 57.85; H, 9.71. Found: C, 58.09; H, 9.87.

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Supplementary Material Available: Experimental procedures for the preparation of the following substances: *trans*-1-(2-(tributylstannyl)-3-cyclohexenyl)-1-ethanone, *trans*-1-(3- and 4-(phenylthio)-6-(tributylstannyl)-3-cyclohexenyl)-1-ethanone, *trans*-2-(4-(phenylthio)-6-(tributylstannyl)-3-cyclohexenyl)-2-propanol, 7,7-dimethyl-3-(phenylthio)bicyclo[4.1.0]hept-3-ene, *exo*- and *endo*-7-methyl-3-phenylbicyclo4.1.0]hept-3-ene, *trans*-1-(3- and 4-methyl-6-(tributylstannyl)-3-cyclohexenyl)-1-phenylethanol, *exo*- and *endo*-3,7-dimethyl-7-phenylbicyclo[4.1.0]hept-3-ene, and *trans*-3,4-dimethyl-6-(tributylstannyl)-3-cyclohexenecarboxaldehyde (6 pages). Ordering information is given on any current masthead page.

Enzymatic and Chemical Oxidations of Leurosine to 5′-Hydroxyleurosine

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The major product obtained when leurosine was oxidized by microorganisms, copper oxidase enzyme systems, benzoquinone, and DDQ has been identified as a mixture of chromatographically inseparable 5′-hydroxyleurosine isomers. The leurosine oxidation product is only one of several possible positional carbinolamine isomers. The precise location of the carbinolamine functionality was clearly determined by carbon-13 NMR spectral analysis of 5′-deuterioleurosine obtained by sodium borodeuteride reduction of the carbinolamine. By virtue of the oxidants employed, it is likely that leurosine is first oxidized to a nitrogen centered cation-radical which loses hydrogen to form an iminium derivative that adds water. The possible occurrence of equilibrium mixtures of isomeric iminium intermediates in the oxidation reaction mixture was excluded by spectral analysis of the product formed when leurosine oxidations were conducted in deuterium oxide.

Introduction

Leurosine (1) is the most abundant dimeric antitumor alkaloid isolated from the Madagascar periwinkle (*Catharanthus roseus* G. Don, Apocynaceae, also known as *Vinca rosea* L.), and it is closely related in structure to the clinically used antineoplastic alkaloids vincristine and vinblastine. The Vinca alkaloids have been used in the treatment of human neoplasms for nearly 3 decades. However, surprisingly little is known about the types of oxidative transformations these alkaloids undergo, or of the possible roles that biotransformations play in their mechanism(s) of action and/or dose limiting toxicities.[1-3] Considerable evidence incidates that the Vinca alkaloids and their derivatives are extensively converted to other products in living systems,[4-6] but nothing is presently known about the structural changes which occur or the activities of presumed oxidation products.

Microbial transformations of monomeric Vinca alkaloids led to an understanding of the chemistry and biochemistry of alkaloid biotransformations. Aspidosperma and Iboga alkaloids were readily oxidized by unknown enzymes of a bacterium,[7] copper oxidases (ceruloplasmin, laccases),[8] peroxidase,[9] cytochrome P-450,[10] and chemical mimics of the enzyme systems. For example, vindoline (7) underwent one-electron oxidation[8] leading to the formation of a reactive iminium intermediate,[11] which by intramolecular etherification formed an enamine that isomerized and dimerized. Benzoquinone and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and photochemical oxidations mimicked the enzyme reactions by providing the same types of oxidation products. Among other products obtained by microbiological transformation, vindoline gave high yields of a phenol formed by methyl ether cleavage[12] and a carbinolamine formed by oxidation of 14,15-dihydrovindoline.[13] Work with vindoline was essential in clarifying the chemical and biochemical features of Vinca alkaloid biotransformations and in illustrating the broad range of oxidative reactions possible with these compounds.

These efforts have now been extended to the unsymmetrical dimeric Vinca alkaloid leurosine. Leurosine presented a much more complicated case because it contains both Aspidosperma and Iboga alkaloid systems linked to one another in its dimeric structure. Leurosine was readily converted to a major common product by microbiological, enzymatic, and chemical oxidizing systems. This report details the formation and characterization of the new carbinolamine product 5′-hydroxyleurosine (4) and reveals that in this dimer the Iboga ring system is more susceptible to enzymatic and chemical oxidation.

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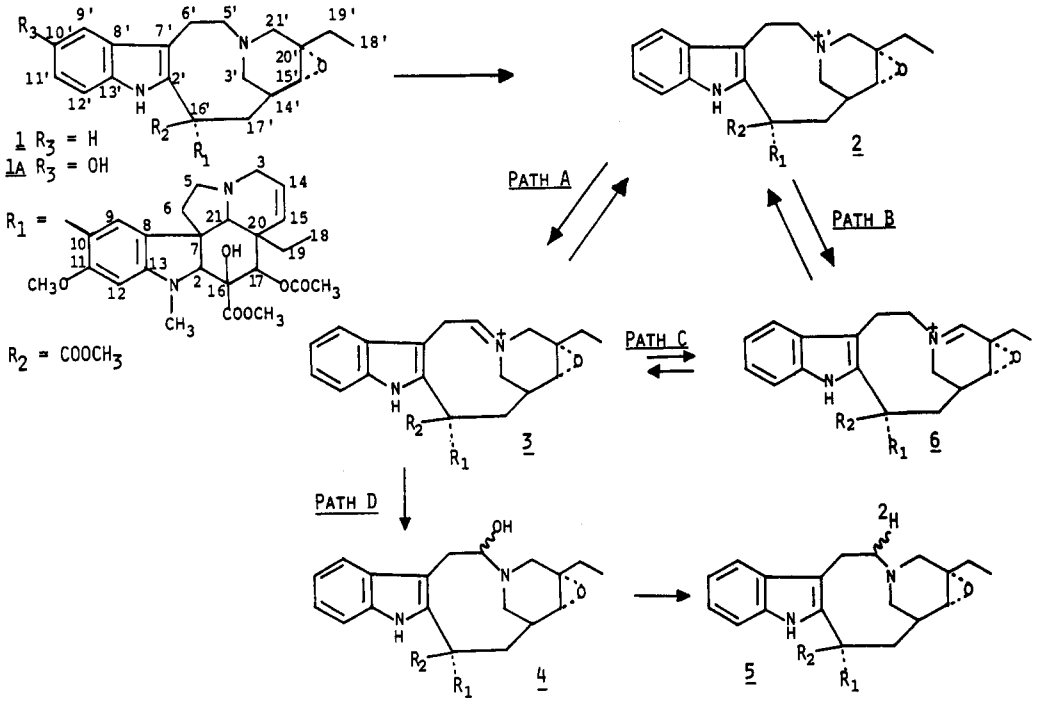

Figure 1. Scheme for the oxidation of leurosine (1) to 5′-hydroxyleurosine (4).

Results and Discussion

Microbiological transformation experiments resulted in the identification of several cultures capable of reproducibly forming new leurosine derivatives. One of these derivatives was identified as 10′-hydroxyleurosine (**1a**), the major oxidation product formed by aromatic hydroxylation of leurosine by Streptomycetes.[14] Recent evidence shows that Streptomycetes contain cytochrome P-450 monooxygenases which may be responsible for the observed leurosine hydroxylation reaction.[15] Two Aspergilli reproducibly gave a different, less polar leurosine product which was difficult to isolate. This prompted the exploration of other enzymes and chemical oxidants to facilitate its production, isolation, and characterization. Copper oxidases[8,11] gave good yields of the Aspergillus leurosine oxidation product (HPLC, TLC), and attention was then focused on this group of biocatalysts.

Copper oxidases are widely distributed in nature and they include fungal and plant laccases and ceruloplasmin which is found in human serum. These copper-containing proteins catalyze the oxidations of phenolic and nitrogen heterocyclic compounds, and their properties have been reviewed in detail.[8,11,16] As with other metalloenzymes, copper atoms that comprise the catalytic centers of the copper oxidases are wrapped within a unique protein environment which potentiates their efficiencies as catalysts and confer specificities toward various substrates.[17] The copper oxidases bind organic substrates which serve as the sources of electrons in the reduction of molecular oxygen. The resulting oxidized products are then released from the enzyme active site into the surrounding reaction medium where they may accumulate or catalyze further chemical oxidation reactions. In effect these "shuttle oxidants" transmit the oxidative/reductive power of the enzyme active site to one which is external to the enzyme. Examples of such shuttle systems are found with the peroxidases (ligninases[18,19]) and with the copper oxidases. A large number of "shuttle oxidants" have been described for the copper oxidases, including hydroquinone, other phenols, and phenothiazines.[8,20-22]

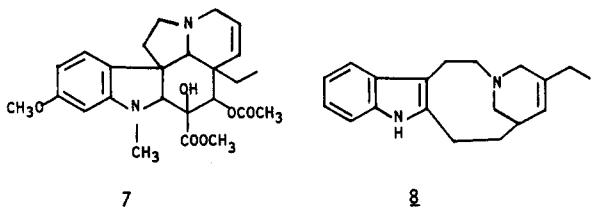

Figure 2. Structures of vindoline (7) and cleavamine (8).

Leurosine was readily transformed by laccase and ceruloplasmin but only in complete reaction mixtures containing the copper oxidases, alkaloid, and catalytic amounts of "shuttle oxidants" including chlorpromazine, phenylenediamine·HCl, syringaldazine, L-DOPA, *N,N*-dimethyl-*p*-phenylenediamine·HCl, or hydroquinone. Histamine·HCl, harmol·HCl, or harmine had no influence on copper oxidase oxidation rates of leurosine. All of the compounds mentioned are known substrates with ceruloplasmin[20] and are directly oxidized by the enzyme to unstable and reactive radical intermediates. In essence, the shuttle oxidants undergo cyclic oxidation/reduction, and the cycle is driven in the presence of any compound—like leurosine—with suitable oxidation potential.

Leurosine is not a direct substrate for the copper oxidases, and it is incapable of interacting at the enzyme active site. With complete reaction mixtures, leurosine sulfate stimulated oxygen uptake at rates of 0.37 μM/

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min/unit of ceruloplasmin and 270 μM/min/unit of laccase. Under identical reaction conditions,[8,22] vindoline (7) and cleavamine (8) oxidation rates were measured at 0.14 and 0.50 μM/min/unit of ceruloplasmin. With laccase and ceruloplasmin, preparative-scale enzyme reactions gave 30–40% yields within 6 h, and all of the leurosine sulfate was consumed within 24 h.

These observations led us to develop a simple chemical mimic of the copper oxidase enzyme oxidation reaction. Reaction of leurosine with an excess of benzoquinone resulted in the rapid formation of the same compound formed by Aspergilli and by the copper oxidases. Reactions were optimal under neutral conditions and were very sluggish when the reaction medium was slightly acidic (pH 4.4), thus suggesting that the lone pair of electrons on nitrogen was required in the alkaloid oxidation reactions. Yields of the leurosine oxidation product were the same when reactions were conducted in air or under an atmosphere of argon gas, thus ruling out the direct participation of molecular oxygen in leurosine oxidations. This also indicated that the carbinolamine oxygen atom derived from water. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) also catalyzed the reaction in nonaqueous solvents. With DDQ, a better oxidizing agent than benzoquinone, reactions were most rapid in benzene solution, but they were also excellent when conducted in methanol. Since DDQ oxidations were conducted in nonaqueous solvent media, leurosine was most probably converted to an iminium species like 3 or 6 (Figure 1), and the water necessary to form the carbinolamine product arose during reaction workup or during chromatography. The facile and high yielding reactions observed with DDQ render it somewhat surprising that this mild reagent has found little general use in nitrogen heterocyclic chemistry.

Since microbial, enzymatic, and chemical systems all produced the same leurosine oxidation product, 1,4-benzoquinone was used to produce relatively large amounts of it for complete spectral and chemical analyses. After chromatographic purification, 4 was obtained in 57% yield. The UV spectrum of the oxidation product was essentially the same as the spectrum of leurosine, indicating that the chromophoric centers remained unchanged. The field desorption mass spectrum gave a molecular ion at m/z 824.39705 (vs. 808 for leurosine) while the electron impact spectrum gave m/z 807.4022 as the peak of highest mass (M – OH). These observations suggested the incorporation of an oxygen atom into the structure of the leurosine product. The 360-MHz proton NMR spectrum of 4 was similar to that of leurosine[23] and provided little direct structural information of importance. Decoupling of the signal at 1.22 ppm (H-14′) resulted in a slight change in the splitting pattern of the signal at 3.04 ppm for proton H-3′A, and decoupling of the signal centered at 3.32 ppm (H-3A) resulted in the collapse of the doublet of doublets at 5.86 ppm (H-14) to a doublet. These results enabled the exclusion of positions 3′ or 3 as the possible sites for structural change.

Carbon-13 NMR spectral comparison of the leurosine oxidation product vs. leurosine revealed great similarities in signals for all of the carbon atoms of the Aspidosperma portions of the two compounds.[24] Missing from the spectrum of the new compound was a methylene carbon signal normally resonating at 49.84 ppm for the aminomethylene group at position 5′ of the Iboga half of the molecule. In its place were two methine carbon signals resonating at 89.50 and 87.98 ppm consistent with the presence of isomeric carbinolamine functional groups in the new leurosine stucture.[13,25] The presence of several "twinned" peaks for carbon atoms 3′, 5′, 6′, 7′, 14′, 15′, 18′, 19′, 20′, and 21′ indicated that the carbinolamine functional group was at either position 5′ or 21′ of the leurosine structure. Confirmation of the presence of a carbinolamine functional group was obtained by treatment of 4 with sodium cyanoborohydride in acidic methanol which resulted in its complete reduction back to leurosine (mass spectrum, chromatography).

The structure of the carbinolamine as 4 was unambiguously established by introducing a deuterium label directly at the carbinolamine site and by carbon-13 NMR spectral analysis of the deuteriated product. Reduction of 4 with *sodium borodeuteride* in deuteriomethanol (CH_3OD) afforded monodeuterioleurosine (5) in good yield. The carbon-13 NMR spectra of leurosine (1) and 5′-deuterioleurosine (5) were essentially superimposable except for the carbon signal for C-5′. This was the only carbon signal absent in the spectrum of 5. The presence of deuterium at C-5′ in 5 causes the carbon signal at this deuteriated position to completely disappear under normal proton-decoupled, broad-band carbon spectral acquisition conditions. This spectral occurrence[26–28] is the result of several coincidental phenomena including the increased multiplicity of C-5′ due to deuterium/carbon coupling, the increased relaxation time of the deuterated carbon signal, and the loss of a NOE which normally results in enhanced carbon signals. Thus, this very direct method of "tagging" a carbinolamine position with deuterium makes it possible to unambiguously assign the location of the original carbinolamine functional group by routine carbon-13 NMR spectroscopy. Deuterium NMR analysis of the labeled compound 5 could also provide useful information, but the body of knowledge of deuterium NMR spectroscopy of dimeric Vinca alkaloids is very sparse, and the low sensitivity of deuterium NMR vs. carbon-13 NMR would require unreasonably long spectral acquisition times. The technique described here presents a powerful means of indirectly identifying the position of unstable carbinolamine/iminium intermediates.

It is possible to postulate a scheme by which 5′-hydroxyleurosine is formed (Figure 1). The most likely first intermediate is the cation radical (2) which eliminates a proton and an electron by either path a or b to form iminium intermediates 3 or 6, respectively. Direct addition of water to 3 would provide 5′-hydroxyleurosine. If 6 forms first, the initial iminium intermediate would either have to revert back to 2 or undergo direct conversion to 3 (path c) before adding water (path d). Path c, involving a concerted intramolecular 1,3-hydrogen shift is thermally forbidden, but this could be accommodated via an intermolecular process. The possible existence of paths b and c were examined by conducting leurosine oxidation in deuterium oxide. If either of these pathways occurs, deuterium from the reaction medium would be incorporated at position C-21′ of 4. Carbon-13 NMR and mass spectral analysis of leurosine obtained by *sodium borohydride* reduction of carbinolamines generated in heavy

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water ruled out the presence of deuterium and thus provided evidence that the 5'-iminium derivative (3) is formed directly by path a.

This work clearly demonstrates the susceptibility of the 5'-position of the Iboga alkaloid half of leurosine to oxidation by an array of microbial, enzymatic, and chemical oxidants. Surprisingly, no evidence was obtained to indicate that similar reactions occurred with the vindoline half of the leurosine structure. Thus, all observed transformation reactions only occur on the upper or Iboga half of this dimer. As observed before in our work with vindoline,[8,9,11] the production of reactive cation radical, iminium, and carbinolamine intermediates of the Catharanthus alkaloid may have toxicological significance due to their suceptibilities to nucleophilic attack. Bioassay of the carbinolamine (4) revealed that the compound is inactive vs. the in vitro CCRF-CFM cell culture system at concentrations of 0.02–20 μg/mL.[29,30] This result and the finding that 4 exhibits almost no oxygen uptake when oxidized by the copper oxidases strongly suggests that 4 is the end product of the oxidation pathway and that it is recalcitrant to further biochemical or chemical transformation.

These findings suggest that dimeric Vinca alkaloids like leurosine are subject to a common pathway of oxidative transformation by chemical and biochemical catalysts. Unexpectedly, oxidations of leurosine using horseradish peroxidase[9] or photochemical or electrochemical oxidizing systems afford mixtures of products which are chromatographically different from 4. This suggests that dimeric alkaloid oxidations are not simply a matter of standard oxidation potentials but that other factors enter into reactions to direct oxidations toward different functional groups and transformation pathways. This result is different from that obtained when the monomeric alkaloid vindoline was subjected to chemical, photochemical, and enzymatic oxidations to yield exactly the same oxidation products in each case.[8,9,11] The identification of leurosine oxidation products obtained by peroxidase, photochemical, and electrochemical oxidations are in progress.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas Hoover melting point apparatus and are uncorrected. IR spectra (KBr) were recorded on a Beckman IR 4240 spectrophotometer. UV spectra were determined in 95% ethanol solution on a Phillips Pye Unicam SP 1800 spectrophotometer. Proton (H-1) and carbon (C-13) nuclear magnetic resonance spectra were recorded in deuteriochloroform solution with a Bruker WH-360 FT spectrophotometer operating at 360.134 MHz for H-1 and 90.556 MHz for C-13 by using Me_4Si (δ = 0) and deuteriochloroform (δ = 77.0) as internal references. Low resolution EI (70 eV) and CI (ammonia as the reagent gas) mass spectra were obtained by using a Nermag-S-a spectrometer, while high resolution mass spectra were obtained on a Kratos AEI MS-50 instrument at Eli Lilly and Company and by the Midwest Center for Mass Spectrometry. Optical rotations of compounds dissolved in chloroform solution were determined with a Perkin-Elmer 141 polarimeter.

Leurosine sulfate was obtained as a generous gift from Eli Lilly and Co., Indianapolis, IN. Before use, the compound was fully characterized by proton and carbon-13 NMR, and its purity was verified by TLC and HPLC.

Chromatography. Thin-layer chromatography (TLC) was performed on 0.25 or 0.5 mm thick silica gel GF_{254} (E. Merck) plates which were oven-activated at 110 °C for 30 min prior to use. Solvent systems were A, benzene/methanol (5:1); B, ethyl acetate/methanol/dichloromethane (1:1:1); or C, chloroform/methanol (15:1). With these chromatographic systems, leurosine gave R_f values of 0.42, 0.70, and 0.65; while 5'-hydroxyleurosine gave R_f values of 0.50, 0.82, and 0.72, respectively. Spots were visualized by UV fluorescence quenching at 254 nm, and by spraying developed plates with Dragendorff's reagent.

Column chromatography was performed using silica gel (60–200 mesh, Baker 3405) which was oven-activated at 110 °C for 30 min prior to use.

High performance liquid chromatography (HPLC) was performed with a Waters Associates ALC/GPC 202 instrument equipped with a Waters M6000a pump, a U6K Universal injector, and a 254-nm differential UV detector. Alkaloid separations were achieved at a nominal flow rate of 1 mL/min at operating pressures of 1500 psi by using the following systems: A, a C-18 column (Altech, 0.625 × 25 cm) eluted with MeOH/0.005 M $(NH_4)_2HPO_4$ (4:1); B, with CH_3CN/0.005 M $(NH_4)_2HPO_4$ (3:2); C, a microbondapak phenylcolumn (Altech, 0.4 × 30 cm) eluted with CH_3CN/0.005 M $(NH_4)_2HPO_4$ (1:1); or D, with CH_3CN/0.005 M $NH_4H_2PO_4$ (1:1). In these systems, retention volumes of leurosine were 9.9 mL, 20.0 mL, 23.4 mL, and 29.2 mL and 5'-hydroxyleurosine retention volumes were 12.0 mL, 9.6 mL, 28.0 mL, and 38.9 mL, respectively. The identities of products formed in chemical and biochemical reaction mixtures were confirmed by co-HPLC and co-TLC with authentic compounds. These chromatographic systems enabled the rapid monitoring of reactions.

Biocatalysts. The extracellular laccase of *Polyporus anceps* was prepared by fermentation of the fungus as previously reported by this laboratory.[16] Human ceruloplasmin (type III) was purchased from Sigma Chemical Co., with an expressed activity of 5400 units/mL in pH 7.0, 0.25 M sodium chloride, 0.05 M sodium acetate buffers. Units of enzyme activity for laccase[16] and ceruloplasmin[8] have been previously defined.

Aspergillus terricola (UI-AT) and *Aspergillus ochraceous* (NRRL-398) were both used in microbial transformation experiments with leurosine sulfate as substrate.

Enzyme Reaction Procedures. Enzyme reactions with ceruloplasmin and laccase were conducted at 37 °C and 27 °C, respectively, as previously described.[8,16] Leurosine sulfate (4.5 mg, 5 × 10^{-6} mol) was added to enzyme mixtures in 0.1 mL of methanol. Samples of 1.0 mL of enzyme reactions were withdrawn at various time intervals, adjusted to pH 10 with 28% ammonium hydroxide, and extracted with 1 mL of ethyl acetate, and the extracts were examined by TLC and HPLC.

Rates of oxidations were determined by measuring oxygen uptake by using a Clark electrode[8] connected to a temperature-controlled, stirred reaction vessel of 3.73 mL. Neither of the copper oxidase enzymes would oxidize leurosine sulfate in the absence of a shuttle oxidant. "Shuttle oxidants" were employed in molar ratios of 1:5 vs. leurosine sulfate and included 3-hydroxy-4-methoxyphenethylamine, harmine, harmol hydrochloride, histamine, levodopa, syringaldazine, hydroquinone, *N,N*-dimethyl-*p*-phenylenediamine, and chlorpromazine. Controls consisted of enzyme alone, mixtures containing only organic substrates plus shuttle oxidants, and complete incubation mixtures with boiled enzymes. No reactions were observed when boiled enzymes were used or when enzymes were absent from reaction mixtures.

Microbial Transformation of Leurosine to 5'-Hydroxyleurosine by *Aspergillus terricola* and *A. ochraceous*. A standard two-stage fermentation protocol[31] was used in the cultivation of microorganisms. Leurosine sulfate (25 mg) was dissolved in 0.2 mL of dimethylformamide, and the solution was divided equally between two DeLong culture flasks (125 mL) each holding 25 mL of 24-h-old stage II cultures of *A. terricola* (UI AT) or *A. ochraceous* (NRRL 398). Reaction flasks were harvested 72 h after addition of leurosine sulfate, adjusted to pH 9.0 with 28% ammonium hydroxide, and extracted with equal volumes of ethyl acetate. After concentration, yields of 5'-hydroxyleurosine were estimated by HPLC (system B) and TLC (system A) to be 35–50% for the two organisms. Control reactions consisting of

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cultures without leurosine sulfate, and flasks containing the alkaloid in buffers held at pH 3.6 (0.1 M potassium phthalate), pH 6.9 (0.1 M potassium phosphate), and pH 9.5 (0.1 M sodium borate) demonstrated that no reactions occurred without growing cultures.

Reaction of Leurosine (1) with Benzoquinone in Phosphate Buffer: Synthesis of 5′-Hydroxyleurosine (4). Leurosine sulfate (250 mg) was added to a suspension of benzoquinone (750 mg) in 0.2 M potassium phosphate buffer (pH 7.0, 250 mL) and the mixture was shaken at 250 rpm at room temperature for 2 h. TLC analysis (system A) of the reaction mixture indicated that all of the leurosine had been consumed in the reaction with the accumulation of a single Dragendorff-positive spot. The mixture was adjusted to pH 2.0 with 5% HCl and extracted exhaustively with ethyl acetate (3 × 250 mL) to remove benzoquinone and hydroquinone formed in the reaction. The aqueous layer was then adjusted to pH 10.0 by the addition of 28% NH_4OH and extracted with ethyl acetate (3 × 250 mL), and the ethyl acetate layer was dried over anhydrous sodium sulfate and filtered. Evaporation of ethyl acetate afforded a brownish solid (0.4 g) which was subjected to silica gel column chromatography (65 g, 29 × 3 cm) using benzene/methanol (95:5) as eluent. Elution volumes 258–357 mL afforded pure 5′-hydroxyleurosine (4, 129 mg) as a light-tan solid, elution volumes 410–486 mL gave unreacted leurosine (18 mg), while the intermediate volumes gave mixtures of these two compounds (17 mg).

Characterization of the Benzoquinone Oxidation Product as 5′-Hydroxyleurosine (4). The solid exhibited the following properties: mp 211–214 °C; $[\alpha]^{24}_D$ +92.49° (c, 0.65); UV, nm (log ϵ) 214 (4.64), 262 (4.11), 289 (4.09), 297 (4.03); IR (KBr) cm^{-1} 3458, 2952, 1732, 1606, 1230; ^1H NMR δ 0.835 (3 H, t, J = 6 Hz, H-18), 0.965 (3 H, t, J = 7 Hz, H-18′), 2.114 (3 H, s, 17-$OCOCH_3$), 2.732 (3 H, s, NCH_3), 3.618 (3 H, s, 11-OCH_3), 3.805, 3.820 (6 H, two s, 16-$COOCH_3$, 16′-$COOCH_3$), 5.323 (1 H, d, J = 10Hz, H-15), 5.471 (1 H, s, H-17), 5.844–5.884 (1 H, m, H-14), 6.124 (1 H, s, H-12), 6.623 (1 H, s, H-9), 7.086–7.278 (3 H, m, H-10′, 11′, 12′), 7.489 (1 H, m, H-9′), 8.007 (1 H, indole-NH); ^{13}C NMR, carbon signals for the Aspidosperma portion of the product were nearly identical with those reported for leurosine,[24] and those for the Iboga half of the molecule were as follows, C-2′ (131.30), C-3′ (38.78, 39.29), C-5′ (87.98, 89.50), C-6′ (27.29, 27.36), C-7′ (115.94, 116.05), C-8′ (128.92), C-9′ (117.97), C-10′ (122.21), C-11′ (118.88), C-12′ (110.40), C-13′ (134.58), C-14′ (32.29, 32.39), C-15′ (60.24, 60.35), C-16′ (55.03), C-17′ (30.64), C-18′ (8.48, 8.55), C-19′ (28.49, 28.83), C-20′ (60.99, 61.29), C-21′ (53.76, 54.27), 16′-$COOCH_3$ (173.94), 16′-$COOCH_3$ (53.01); FD-MS, m/z 824.39705 (calcd for $C_{46}H_{56}N_4O_{10}$ 824.39965); EI-MS 807.4022 (M − OH), 806.3852 (M − H_2O), 788.3812 (M − $2H_2O$).

Reduction of 5′-Hydroxyleurosine (4) with Sodium Cyanoborohydride. A 50-μL sample of 6 N HCl and 10 mg of sodium cyanoborohydride were added to a solution of 5 mg of 5′-hydroxyleurosine (4) in 1 mL of methanol. After 15 min, the reaction was evaporated to dryness and extracted into 1 mL of ethyl acetate, and the extract was subjected to HPLC (system B) and TLC (systems A, B, C). The sodium cyanoborohydride reduction product exhibited mass spectral and chromatographic behaviors identical with leurosine (1).

Reduction of 5′-Hydroxyleurosine with Sodium Borodeuteride. Sodium borodeuteride (369 mg) was added to a suspension of 83 mg of 5′-hydroxyleurosine (4) in 20 mL of deuteriomethanol (CH_3OD) and the mixture was stirred in a round-bottomed flask equipped with a drying tube at room temperature. After 28 h, an additional sample of 400 mg of sodium borodeuteride and ethyl acetate (5 mL) was added to the reaction mixture. After a total of 70 h, the solvent was removed by evaporation, the residue was treated with 30 mL of water, and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). After drying over anhydrous sodium sulfate, the organic layer was filtered, and solvent was evaporated to afford 75 mg of a white solid. The reaction product was purified by silica gel column chromatography (25 g, 24 × 2 cm) using benzene/methanol (95:5) as eluent. Elution volumes 127–201 mL afforded pure monodeuterated leurosine (5, 39 mg) as a white solid; high resolution mass (FAB), m/z 810.4157 (calcd for $C_{46}H_{55}N_4O_9D$ + H, 810.4188).

Reaction of Leurosine with Benzoquinone in D_2O and Reduction of the Product with Sodium Borohydride. A 0.2 M phosphate buffer solution was prepared by dissolving 1.36 g of KH_2PO_4 in 50 mL of D_2O and adjusting the solution to pH 7.06 with 40% NaOD in D_2O. Leurosine sulfate (201 mg) was dissolved in the buffer, 600 mg of benzoquinone was added, and the mixture was stirred with a Drierite guard tube to prevent the entry of atmospheric H_2O into the system. After 3 h the solution was adjusted to pH 2.0 with 5% HCl and extracted with ethyl acetate (3 × 50 mL) to remove benzoquinone and hydroquinone from the reaction mixture. The aqueous solution was then adjusted to pH 10.0 with 28% NH_4OH and extracted with ethyl acetate (4 × 50 mL). The ethyl acetate was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to yield 200 mg of brown solid. This material was purified on a column of silica gel (60 g, 28 × 3 cm) using benzene/methanol (95:5) as eluent. Elution volumes 232–315 mL afforded 5′-hydroxyleurosine (80 mg). This compound was dissolved in a mixture of 35 mL of methanol and 15 mL of ethyl acetate, and 500 mg of $NaBH_4$ was added. The reaction mixture was stirred for 40 h, the solvent was evaporated, and the residue treated with water (50 mL) and extracted with ethyl acetate (4 × 50 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate and filtered. Removal of solvent afforded pure leurosine (65 mg) which was characterized by TLC (system A), mass spectrometry, and proton and carbon-13 NMR.

Oxidation of Leurosine with Benzoquinone in an Argon Atmosphere. The same reaction was conducted in 5 mL of buffer containing 15 mg of benzoquinone in a 10-mL Thunberg tube under an argon atmosphere. Leurosine sulfate (5 mg) was held in the side arm of the tube while the buffer solution was purged with argon. The reaction was initiated by mixing the contents of the side arm with the buffer solution. After 2 h, the reaction mixture was worked up as described above and subjected to TLC (system A). This reaction and a control reaction conducted in an air atmosphere both gave estimated yields of 50% of 5′-hydroxyleurosine.

Oxidation of Leurosine with DDQ. (a) In Benzene. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2 mg) was added to a solution of 5 mg of leurosine base in 1 mL of benzene, and the mixture was stirred at room temperature. The progress of the reaction was monitored by HPLC (system E) and TLC (system A) to show that leurosine was completely oxidized to 5′-hydroxyleurosine in 15 min.

(b) In Methanol. DDQ (2 mg) was added to a suspension of 5 mg of leurosine in 1 mL of methanol and the mixture was stirred at room temperature. TLC and HPLC analyses (systems A and E, respectively) were used to reveal that the reaction was 50% complete in 15 min and 70% complete in 5.5 h. The sole product was 5′-hydroxyleurosine.

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Supplementary Material Available: Tables of carbon-13 NMR spectral data for 1, 4, and 5 (3 pages). Ordering information is given on any current masthead page.