Oxoaporphine Alkaloids: Conversion of Lysicamine into Liriodendronine and its 2-*O*-Methyl Ether, and Antifungal Activity

Varol Pabuccuoglu[+], Maria Danka Rozwadowska, and Arnold Brossi[*]

Medicinal Chemistry Section, LAC, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, U.S.A.

Alice Clark and Charles D. Hufford

Department of Pharmacognosy, School of Pharmacy, The University of Missippi, University, MS 38677, U.S.A.

Clifford George and Judith L. Flippen-Anderson

Laboratory for the Structure of Matter, Naval Research Laboratory, Department of the Navy, Washington, D.C. 20375, U.S.A.

Received December 7, 1989

Pschorr reaction of diazonium salt **7** in aqueous methanolic sulfuric acid afforded, besides lysicamine **2**, the orange colored sulfate of oxodibenzopyrrocoline (**8**). The structure is fully supported by an X-ray analysis of its picrate salt. Selective ether cleavage of lysicamine (**2**) with 48% HBr afforded a hydrobromide of **9**, and free betaine **9** on treatment with pyridine-water. Both compounds methylated on treatment with etherial diazomethane on nitrogen to give the known 2-*O*,*N*-dimethylliriodendronine (**11**). Liriodendronine (**10**) was obtained from lysicamine (**2**) on heating with pyridine·HBr at 189°C, and treatment with pyridine-water, as a dark violet betaine. Betaine **12** was obtained by heating **11**·HCl to 200°C. The quaternary salts of lysicamine, lysicamine methiodide (**3**) and lysicamine methosulfate (**4**) were comparable in anticandidal activity to liriodenine (**1**), but were not as active as liriodenine methiodide (**13**).

Oxoaporphin Alkaloide: Herstellung von Liriodendronin und dessen 2-Methylether aus Lysicamin. Bericht über die antifungale Wirkung von Oxoaporphinen

Pschorr-Cyclisierung des Diazoniumsalzes **7** führte in wässrig-methanolischer Schwefelsäure neben Lysicamin (**2**) zum orange gefärbten Sulfat des Oxodibenzopyrrocolins **8**. Diese Formel ist durch X-Ray Analyse des Picrates belegt. Bei der selektiven Etherspaltung von Lysicamin (**2**) mit 48proz. HBr wurde das Hydrobromid des Betains **9** erhalten, das freie Betain nach Behandlung mit Pyridin-Wasser. Beide Verbindungen, **9**·HBr und **9**, wurden bei der Behandlung mit etherischem Diazomethan am N methyliert und lieferten das bekannte 2-*O*,*N*-Dimethylliriodenin (**11**). Liriodendronin (**10**) wurde aus Lysicamin (**2**) durch Erhitzen mit Pyridin·HBr auf 189°C erhalten und lieferte in Pyridin-Wasser das dunkelviolette Betain. Das phenolische Betain **12** wurde beim Erhitzen von **11**·HCl auf 200°C erhalten. Die quartären Salze von Lysicamin, das Methoiodid **3** und das Metheosulfat **4**, zeigten mit Liriodenin (**1**) vergleichbare Wirkung *in vitro* gegen *Candida albicans*, waren aber deutlich schwächer wirksam als Liriodeninmethoiodid (**13**).

The oxoaporphine alkaloids, probably derived from the corresponding aporphine alkaloids by oxidation, have repeatedly been reviewed[1a-d]. Earlier reports noted the antifungal activity of selected oxoaporphine alkaloids[2a]. It is of interest to prepare phenolic congeners of liriodenine (**1**) since it can be speculated that these may be better bioavailable and may even represent putative metabolites of (**1**). We, therefore, decided to prepare phenol **9** and catechol **10** from the well known alkaloid lysicamine (**2**). Both **9** and **10** are present in solution above pH 6.5 as betains and are shown here as such. Lysicamine (**2**) has been synthesized several times from readily available isoquinolines[4-6]. The sequence of reactions starting with amine **6**, prepared by reduction of nitroisoquinoline **5** and *Pschorr* cyclization of diazonium compound **7** is described here in detail since the characterization of **6** has never been performed. The orange sulfate, which separated after *Pschorr* reaction and concentration of the methanolic acidic solution, was never reported and will also be described. The oxodibenzopyrrocoline structure **8** was confirmed by an X-ray analysis of a picrate salt and is included here. Lysicamine (**2**), was obtained after usual workup and crystallization from 95% ethanol in 10-15% yield, and was also used to prepare methoiodide **3** and methosulfate **4**, which, on Si-gel, are considerably more polar than **2** or **9** or **10** (EtOH/Py/NH_4OH = 6/3/1). The insolubility of oxoaporphines in commonly used solvents, particularly phenolic congeners **9** and **10** described later, was a serious drawback in the chemical analysis of these compounds. With the finding that hydrobromides of demethylated oxoaporphines converted with pyridine-water into highly colored betaines, which moved well on Si-gel, helped greatly in accomplishing our objective. *O*-Demethylation of lysicamine (**2**) in 48% HBr at 78°C afforded an orange hydrobromide, which on treatment with pyridine-water, converted into the dark violet betaine **9**. The UV-spectrum of **9**, and its IR-spectrum, which no longer showed

[+]Visiting Fellow from the Faculty of Pharmacy, Ege University, Bornova-Izmir, Turkey.

  0365-6233/91/0101-0029 $ 3.50+.25/0

a carbonyl absorption at 1650 cm^{-1}, are reminescent of *N*-methylated betaines such as **11**[3,8,9] which was obtained on refluxing **2** with methyl iodide[8,9,10]. Methylation of **9** or **9**·HBr, in methanol with etheral diazomethane afforded betaine **11** identical with the reported material[8]. *O*-Demethylation of the hydrochloride of **11** by heating it to 200°C afforded **12**. Full demethylation of **2** in 48% HBr was difficult to accomplish, and **10** could much easier be prepared with pyridine hydrobromide at 189°C. The material obtained after treatment with pyridine-water is the oxoaporphine alkaloid liriodendronine (**10**), isolated from the heart wood of the tulip tree (*Liriodendron tulipifera*)[9].

Betaines of the oxoaporphine series and their hydrobromides are methylated with diazomethane on nitrogen, and not on the charged oxygen atom. Structure of the orange salt, obtained as water soluble sulfate in the *Pschorr* reaction of **7** was suggested to be that of the dibenzopyrrocoline ketone **8** on the basis of MS m/z = 292, a C=O-frequency at 1715 cm^{-1}, and a ^{1}H-NMR spectrum which showed 8 aromatic protons and 2 methoxy groups. This structure is related to that of a tetrahydro analog[11]. Treatment of a solution of the orange sulfate of **8** in 2N-NaOH, followed by acidification, did not lead to any identifiable products. Reduction of **8** sulfate in ethanol (PT), or with $ZN/CH_3OH/NH_4Cl$ in refluxing methanol, or with $NaBH_4$ in methanol, led to complex mixture of products which were highly sensitive to air-oxidation. Compound **8**, on the other hand, is quite stabile to acid and can be recovered unchanged from 10% H_2SO_4 after heating to 60°C. We decided, for these reasons, to confirm the unusual structure **8** formed along with **1** in the *Pschorr* reaction of **7** by an X-ray analysis. The orange sulfate afforded, with a solution of picric acid in 60% acetic acid, a dark yellow picrate, which after crystallization from 60% acetic acid, was found suitable for this purpose.

X-Ray Analysis of Oxodibenzopyrrocoline (**8**) *as Picrate*

$C_{18}H_{14}NO_3^+ \cdot C_6H_2N_3O_7^-$, mol. wt. 529.4; monoclinic, space group $P2_1/c$; a = 10.462 (1), b = 15.341 (3), c = 13.830 (3) Å, β = 91.51 (1)°, V = 2218.7 (6) $Å^3$, Z = 4, d_{calc} = 1.56 gm/cm^3, μ = 1.01 mm^{-1}. Measurements were obtained with a Nicolet R3m/V automatic diffractometer using Cu Kα radiation (λ = 1.54178 A) with an incident beam graphite monochromator. The 2807 independent reflections were reasured at room temp. using the θ/2θ scan technique with a variable scan rate out to $2\theta_{max}$ = 112.0°. Data were corrected for *Lorentz* and polarization effects, but absorption effects were ignored. The structure was solved by direct methods and refined by full-matrix leat-squares techniques (non-H atoms anisotropic, H atoms isotropic) using the 2508 reflections for which $F_o > 3\sigma(F_o)$ to a final R factor of 0.060, R_w = 0.084. The function minimized by the least-squares was $\Sigma W(|F_o|-|F_c|)^2$ where $w = 1/[\sigma^2(\sim F_o|)^2+g(F_o)^2]$ and g = 0.00023. The goodness of fit parameter was 1.54. All calculations were carried out with the SHELXTL system of programs[12].

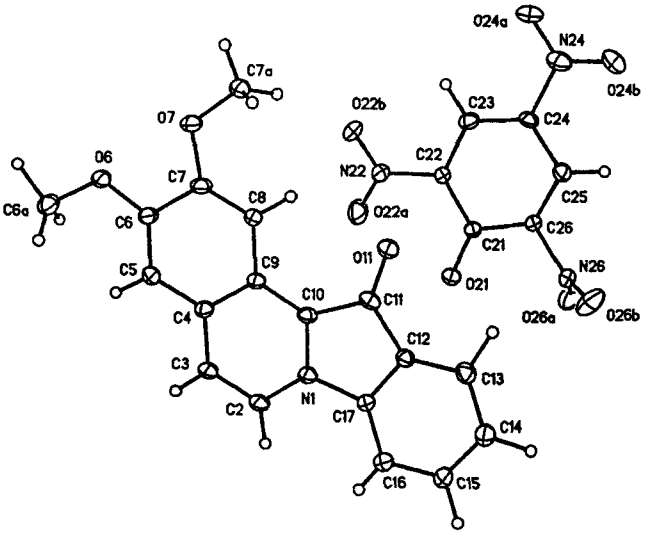


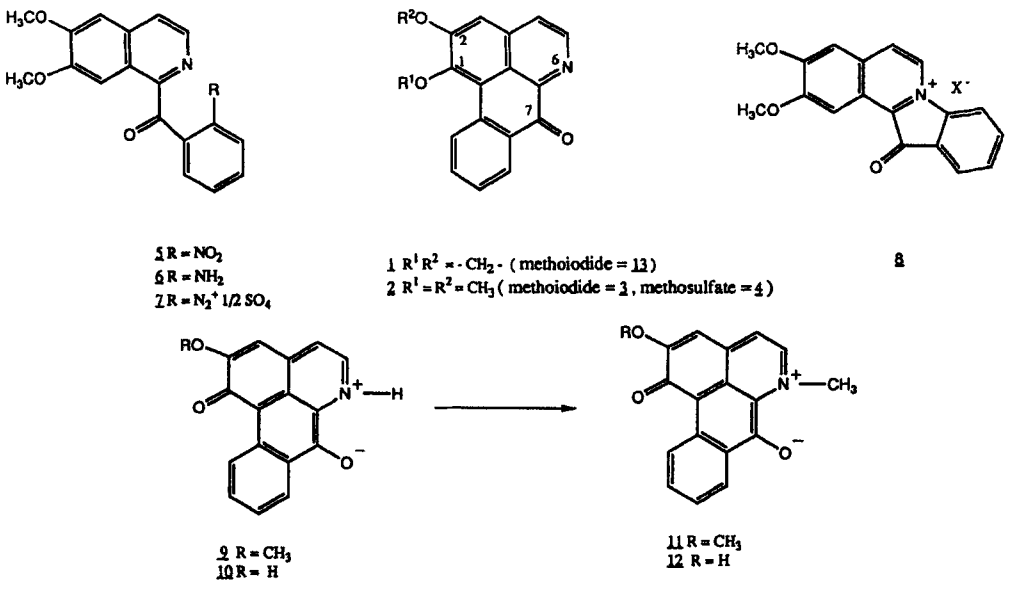
Figure 1: Diagram showing the structure and conformation of the picrate of **8**. It is drawn using the experimentally determined coordinates with thermal parameters at the 20% probability leve.

Discussion

Figure 1 shows the results of the X-ray study on **8**. Tables of coordinations, bond length, and angles have been deposited with the Cambridge Crystallographic Data Base[13]. Both the picrate ion (excluding the oxygen atoms) and the pyrrocoline moiety are essentially planar. Of the three nitro groups on the picrate ion only one is significantly rotated out of the ring plane and it is approximately perpendicular to it (C21-C26-N26-O26a torsion is 69.5°). For the remaining nitro groups the equivalent torsion angles are -17.9 and -8.3°. The fused ring system of **8** is fully conjugated with bond lengths ranging from 1.343(6) - 1.491(6) Å. The three non-shared ring bonds in the five-membered ring (N1-C17, C11-C10 and C11-C12 at 1.453(4), 1.491(4) and 1.468(4) Å, respectively) and C6-C7 at 1.455(4) Å are significantly longer than normal conujugated bonds (average value for the remaining 14 bonds is 1.378(4) Å). This is due to the strain put on the five-membered ring in the planar ring system and to repulsive forces between O6 and O7. Packing in the crystal consists of a system of parallel planes containing both picrate and pyrrocoline ions arranged in such a way that the pyrrocoline is surrounded by symmetry related pyrrocoline molecules and picrate ions within a layer and only by the oppositely charged picrate ions in neighboring layers (see figure 2).

Antifungal Activity

The anticandidal activity of laysicamine (**2**), lysicamine methiodide (**3**), lysicamine methosulfate (**4**), betaine **9**, and liriodendronine (**10**) in comparison with liriodenine (**1**) and its methiodide (**13**) are summarized in Table 1. Lysicamine (**2**), which differs from the active oxoaporphine liriodenine (**1**)[2a,2b] only in the substituents at positions 1 and 2 (OCH_3 versus O-CH_2-O), does not demonstrate any measureable



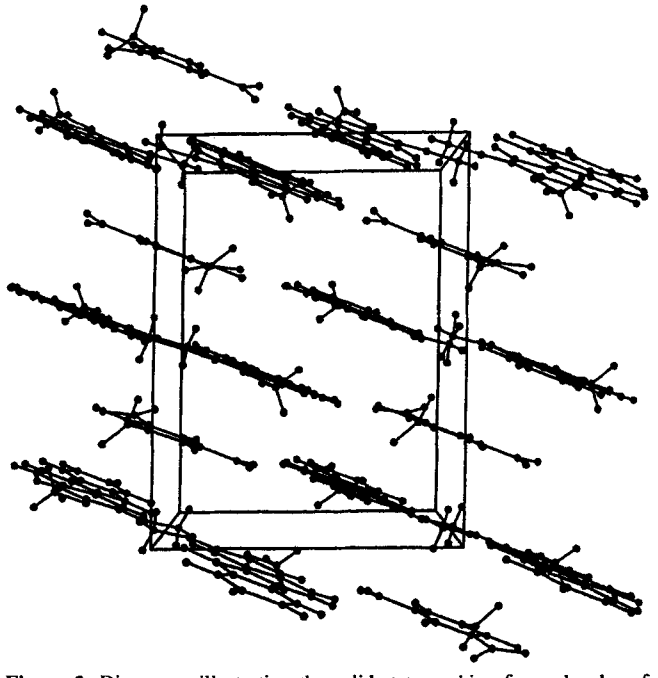


Figure 2: Diagramm illustrating the solid state packing for molecules of the picrate of **8**.

activity (Table 1). Thus, it would appear that the presence of the methylene dioxy in ring A is necessary for activity in the free base. Conversion of lysicamine (**2**) to its quaternary methiodide (**3**) or methosulfate salt (**4**) leads to a considerable improvement in activity (Table 1), a trend previously observed with other oxoaporpholines[2a]. Thus, it can be concluded that quaternization of the oxoaporphine alkaloids results in improved activity against the yeast *C. albicans*. The betains **9** and **10** showed no activity against *C. albicans*. Furthermore, quaternization alone is apparently insufficient to confer activity to the alkaloids, since the quarternary salt **11** has previously been shown to be inactive[2a], as is the parent betaine **9** (Table 1).

Table 1. Anticandidal Activity of Oxoaporphine Alkaloids

Antifungal	Minimum Inhibitory Concentration (μg/ml) *Candida albicans* B311	*Candida albicans* 10231
Liriodenine (**1**)	3.1	6.2[2a]
Liriodenine methiodide (**13**)	1.5	0.4
Lysicamine (**2**)	-	-
Lysicamine methoiodide (**3**)	6.2	0.8
Lysicamine methosulfate (**4**)	12.5	1.5
2-O-Methylliriodendronine (**9**)	50	50
Liriodendronine (**10**)	25	25
Amphotericin B	0.4	0.4

We would like to thank Dr. *Herman J.C. Yeh* from our laboratory for helpful technical advise. We also would like to than Professor *Nelson Leonard* from the University of Illinois at Urbana, who spent a sabbatical year at the NIH as a Fogarty Scholar-in-residence, for his interest in this investigations and his many useful comments. The NRL authors were supported, in part, by the office of Naval Research. This work was supported in part by Contracts NO1-AI-42549 and NO1-AI-72638 from the Division of AIDS, National Instituts of Health, USA.

Experimental Part

TLC: Plates from Analtech Inc. - Melting points: Fischer-Johns appartus, uncorrected. - Ultaviolet spectra: Hewlett-Packard 8450 UV-VIS spectrometer in MeOH (λ max log ε). - IR spectra: Beckman 4230 instrument KBr. - ^{1}H-NMR spectra: Varian-XL-300, tetramethylsilan as internal standard, chemical shifts in δ values (ppm). - Electron impact mass spectra: V.G. Ins. 7070 F at 70 eV.

1-(o-Aminobenzoyl)-6,7-dimethoxyisoquinoline (**6**)

A suspension of **5** (2.45 g, 7.27 mmol) and PtO_2 (0.246 g, 1.08 mmol) in methanol was reduced under H_2 for 2 h. The catalyst was removed by filtration and washed with methanol, and the methanolic solution was evaporated to dryness. The residue was crystalized with 95% ethanol to yield

crystylline **6** (1.44 g, 65%); mp 167-169°C. - UV: 236 (4.45), 240 (4.44), 245 (4.43), 267 (sh), 327 (3.45), 385 (3.67) nm. - IR: 3520; 3370; 1630 cm^{-1}. - ^{1}H-NMR ($CDCl_3$): 3.91, 4.04 (2s; 3H each, $2XOCH_3$), 6.48 (s; 1H, aromat.), 6.52 (d; 1H, aromat., J = 7 Hz), 7.25 (m; 3H,aromat.), 7.59 (d; 1H, aromat., J = 5.6 Hz), 8.44 (d; 1H, aromat., J = 7 Hz). - MS: m/z = 308 (25%, M^+), 280 (60), 279 (100), 263 (15), 249 (5), 234 (10), 221 (5); EIMS for $C_{18}H_{16}N_2O_3$ Calc. 308.1160, Found 308.1146.

Lysicamine (**2**)

Amine **6** (1.29 g, 4.1 mmol) in methanol was chilled in an ice-bath, then 10% H_2SO_4 was added. When the temp. of solution dropped to 0-2°C, 1M $NaNO_2$ solution (7.54 ml) was added dropwise, being careful not to allow the temp. to rise above 5°C. After addition of $NaNO_2$, the solution was stirred for 40 min in an ice-bath and then refluxed for 1 h. The methanolic solution was than concentrated in vacuo to half of its original volume, the red precipitate (**8**) was removed by filtration and washed with cold methanol. The filtrate was concentrated, the aqueous residue was made alkaline with 10% NaOH and extracted with $CHCl_3$ (2 x 75 ml). The chloroform extract was reextracted with 5% HCl. The acidic solution was made basic with 10% NaOH and extracted with $CHCl_3$. The chloroform extract was dried over Na_2SO_4, and evaporated. The residue was crystalized from 95% EtOH to yield greenish-yellow crystals (0.11 g, 10%); mp of 211°C (decomp.). The spectral data (UV, ^{1}H-NMR, IR, EIMS) of **2** are identical with those reported[4-6].

Lysicamine methoiodide (**3**)

Lysicamine (**2**) (50 mg, 0.17 mmol) and CH_3I (3 ml) in acetone (9 ml) were stirred for 2 days. The precipitate formed was filtered to give a crystalline orange methoiodide **3** (50 mg, 67%); mp 140°C (decomp). - UV: 225 (4.71), 238 (4.62), 250 (4.63), 279 (4.58), 376 (3.64), 385 (3.66), 456 (3.44). - IR: 1650; 1620; 1600 cm^{-1}. - ^{1}H-NMR (DMSO-d_6): 4.09, 4.21 (2s; 3H each, $2XOCH_3$), 4.70 (s; 3H, N-CH_3), 7.67 (t; 1H, aromat., J = 7.6 Hz), 7.96 (m; 2H, aromat.), 8.33 (d; 1H, aromat., J = 8 Hz), 8.64 (d; 1H, aromat., J = 8 Hz), 9.00 (d; 1H, aromat., J = 6.7 Hz), 9.03 (d; 1H, aromat., J = 8 Hz). EIMS: for $C_{18}H_{13}NO_3{\cdot}CH_3$ Calc. 306.1130, Found 306.1146.

Lysicamine methosulfate (**4**)

Lysicamine (**2**) (30 mg, 0.1 mmol) in acetonitril (3.5 ml) was refluxed with dimethylsulfate (0.01 ml) for 5 h. Cooling yielded orange crystals of **4** (30 mg, 70%); mp 184-189°C. - UV: 250 (4.14), 278 (4.11), 385 (3.56), 454 (3.22). - IR (KBr): 1660; 1235; 1135 cm^{-1}. - ^{1}H-NMR (DMSO-d_6): 3.57 (br. s; $CH_3OSO_3^-$), 4.09, 4.20 (2s; 3H each, $2XOCH_3$), 4.70 (s; 3H, N-CH_3), 7.76 (t; 1H, aromat., J = 7.4 Hz), 7.99 (s; 1H, aromat.), 8.00 (t; 1H, aromat., J = 7.4 Hz), 8.33 (d, 1H, aromat., J = 8 Hz), 8.64 (d; 1H, aromat., J = 6.6 Hz), 9.00 (d; 1H, aromat., J = 6.6 Hz), 9.03 (d; 1H, aromat., J = 8 Hz), - EIMS: for $C_{18}H_{13}NO_3{\cdot}(CH_3)_2SO_4$ Calc. 306.1130, Found 306.1135.

2,3-Dimethoxy-12,12a-dihydro-12-oxodibenzp[b,g]pyrrocolinium sulfate (**8**)

The orange precipitate obtained in the preparation of **2** crystallized from 10% H_2SO_4 in the form of long hairy orange needles; mp 235°C. - UV: 225 (4.15), 257 (4.52), 299 (4.57), 351 (3.60), 426 (4.03); (MeOH + 0.1N NaOH) = 219 (4.77), 257 (4.58), 320 (4.12) nm. - IR: 1715; 1620; 1290; 1050 cm^{-1}. - ^{1}H-NMR (TFA): 4.20, 4.21 (2s; 3H each, $2XOCH_3$), 7.50 (s; 1H, aromat.), 7.68 (t; 1H, aromat., J = 7.6 Hz), 7.91 (t; 1H, aromat., J = 7.6 H), 8.01 (d; 1H, aromat., J = 8.9 Hz), 9.11 (d; 1H, aromat., J = 8.9 Hz), 8.43 (d; 1H, aromat., J = 6.6 Hz), 8.52 (s; 1H, aromat.), 8.91 (d; 1H, aromat., J = 6.6 Hz). - MS (70 eV): m/z = 292 (100%, M^+), 277 (25), 276 (25), 263 (7), 248 (35), 231 (8), 219 (13); High Res. EIMS = for $C_{18}H_{14}NO_3$ Calc. 292.0973, Found 292.0955. - For $C_{18}H_{14}NO_3$ x 1.1 H_2SO_4 x 1.4 H_2O (425) Calc. C 50.8 H 4.47 N 3.3 S 8.3 Found C 49.8 H 3.88 N 3.2 S 8.1 Picrate salt (from EtOH) = mp 280°C. - For $C_{24}H_{16}N_4O_{10}$ Calc. C 55.3 H 3.09 N 10.8 Found C 55.3 H 3.13 N 10.8. The picrate sample used for the X-ray analysis was prepared in 60% AcOH and crystallized from AcOH and air-dried, mp 280°C.

2-O-Methylliriodendronine (**9**)

Compound **2** (100 mg, 0.34 mmol) in 48% HBr (2 ml) was heated at 78°C for 19 h. After cooling, the precipitate formed was filtered and crystallized from glacial acetic acid to give a pure orange crystaline hydrobromide salt of **9** (62 mg, 64%); mp 160°C. For preparing betaine **9**, the hydrobromide salt (20 mg, 0.072 mmol) was dissolved in pyridine (2 ml) at 85°C, and the solution was left to cool before water (2 ml) was added to give pure violet crystals of **9** (12 mg, 78%) of mp 265-270°C (decomp.). - UV: 243 (4.13), 269 (3.98), 309 (4.20), 424 (3.59), 592 (3.44); (MeOH + 0.1N NaOH) = 244 (4.24), 252 (4.21), 291 (4.31), 322 (sh), 335 (sh), 381 (2.44), 538 (3.95) nm; (MeOH + 0.1N HCl) = 251 (4.28), 286 (4.27), 396 (3.63), 488 (3.47). - IR: 1625; 1605; 1575 cm^{-1}. - ^{1}H-NMR ($CDCl_3$ + TFA 5%): 4.47 (s; 3H, OCH_3), 7.75 (s; 1H, aromat.), 7.81 (t; 1H, aromat., J = 7.7 Hz), 8.11 (t; 1H, aromat., J = 7.7 Hz), 8.64 (d; 1H, aromat., J = 8 Hz), 8.70 (d; 1H, aromat., J = 6.3 Hz), 8.99 (d; 1H, aromat., J = 6.3 Hz), 9.36 (d; 1H, aromat., J = 8 Hz). - MS: m/z = 277 (100%, M^+), 248 (25), 234 (11), 219 (11), 189 (8); High Res. EIMS: for $C_{17}H_{11}NO_3$ Calc. 277.0738, Found 277.0727.

Liriodendronine (**10**)

Lysicamine (**2**) (100 mg, 0.34 mmol) and pyridine hydrobromide (2 g, 12.5 mmol) were heated at 188°C for 20 min. The reaction mixture was added to water (10 ml) and stirred for 10 min. The insoluble material was filterred and dried in an oven at 50°C *in vacuo*. The dried material was dissolved in pyridine (3 ml) at 85°C, and to this solution, water (3 ml) was added to give dark violet crystals (70 mg, 74%); mp 268-272°C (decomp.). - UV: 258 (4.06), 306 (4.10), 432 (3.47), 576 (3.37), 584 (3.36); (MeOH + 0.1N NaOH) = 256 (4.10), 287 (4.04), 404 (3.25), 512 (3.65); (MeOH + 0.1N HCl) = 255 (4.09), 286 (3.99), 307 (sh), 410 (3.47), 502 (3.24) nm. - IR: 1630; 1580 cm^{-1}. - ^{1}H-NMR ($CDCl_3$ + TFA 5%): 7.86 (s; 1H, aromat.), 7.85 (t; 1H, aromat., J = 7.7 Hz), 8.18 (t; 1H, aromat., J = 7.7 Hz), 8.57 (d; 1H, aromat., J = 6.2 Hz), 8.71 (d; 1H, aromat., J = 8 Hz), 8.84 (d; 1H, aromat., J = 6.2 Hz), 9.51 (d; 1H, aromat., J = 8 Hz). - MS: m/z = 263 (100%,M^+), 236 (7), 235 (42), 207 (7), 178 (6), 176 (6), 149 (15); High Res. EIMS: for $C_{16}H_9NO_3$, Calc. 263.0582, Found 263.0582. Preparation of the hydrobromide salt of **10**: betaine **10** (10 mg, 0.038 mmol) was refluxed with 48% HBr (1 ml). The boiling solution was left to cool to give orange-red crystals (9.5 mg, 75%); mp 183-189°C (decomp).

N,O-Dimethylliriodendronine (**11**)

Lysicamine (**2**) (90 mg, 0.3 mmol) and CH_3I (3 ml) were refluxed in acetone (15 ml) for 2 days. To the boiling solution, MeOH (5 ml) was added and the reaction mixture was left to cool to give pure violet crystals (60 mg, 68%); mp 278°C (decomp.). The spectral data were identical with those reported[8,10].

N-Methylliriodendronine (**12**)

Betaine **11** (25 mg, 0.085 mmol) was dissolved in MeOH (10 ml) and acidified with N-HCl and the solvent was evaporated to dryness. The residue was heated at 200°C for 1 h. Crystallization from MeOH gave **12**; mp >300°C. - UV: 270 (3.56), 308 (3.60), 432 (2.95), 508 (3.02), 548 (2.87), 556 (2.87); (MeOH + 0.1N NaOH) = 272 (3.75), 295 (3.67), 358 (sh), 506 (3.45) nm; (MeOH + 0.1N HCl) = 254 (3.63), 286 (3.59), 336 (2.91), 400 (2.99), 484 (2.76), 496 (2.74), 502 (2.73). - IR (KBr): 1630; 1610; 1580 cm^{-1}. - ^{1}H-NMR (TFA): 4.89 (s; 3H, N-CH_3), 7.71 (m; 2H,

aromat.), 7.98 (t; 1H, aromat., J = 7.3 Hz), 8.37 (d; 1H, aromat., J = 6.4 Hz), 8.44 (m; 2H, aromat.), 9.26 (d; 1H, aromat., J = 8 Hz). - MS (70 eV): m/z = 277 (100%, M^+), 276 (17), 267 (17), 249 (85), 235 (35), 178 (25), 163 (35); - High Res. EIMS: $C_{17}H_{11}NO_3$ Calc. 277.07389, Found 277.0743.

Antimicrobial Evaluation

Initial anticandidal screening was performed according to the general qualitative assay first described by *Hufford*, et al.[2a] as modified by *Clark*, et al.[14,15] and using *Candida albicans* (ATCC 10231) and *C. albicans* (NIHB311) as test organisms.

Culture plates (15 x 100 mm) for the qualitative assay were prepared form 25 ml of *Sabouraud*-dextrose agar. Using sterial cotton swabs, the plates were streaked with a suspension of *C. albicans* (10^6 CFU/ml) prepared as described[15]. Cylindrical plugs were removed from the agar plates by means of a steril cork borer to produce wells with a diameter of approximately 11 mm. To the well was added 100 µl of a 1 mg/ml solution of test compound. The anticandidal activity was recovered as the width (in mm) of the zone of inhibition measured from the edge of the agar well to the edge of the zone after 24 h incubation. Amphotericin B was included (at a concentration of 1 mg/ml) as positive control.

The method used to determine the minimum inhibitory concentration (MIC) was the twofold serial broth dilution assay[14,15] in yeast nitrogen broth (Difco). Using a calibrated sterile inoculating loop, each tube was inoculated with 10 µl of a suspension of *C. albicans* (10^6 CFU/ml)[14]. The MIC value was taken as the lowest concentration of compound that inhibited growth after 24 h incubation period (37°C). The antifungal agent amphotericin B was included as positive control.

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