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APORPHINE ALKALOIDS AND LIGNANS FORMED IN RESPONSE TO INJURY OF SAPWOOD IN *LIRIODENDRON TULIPIFERA**

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Key Word Index—*Liriodendron tulipifera*; Magnoliaceae; discolored sapwood; aporphine alkaloids; (+)-lirioferine; (+)-liriotulipiferine; syringalignans.

Abstract—Two new phenolic aporphine alkaloids, (+)-lirioferine and (+)-liriotulipiferine, were isolated from discolored sapwood of *L. tulipifera*. Injury to the tree stem greatly stimulated biosynthesis of glaucine and phenolic alkaloids related to glaucine including thaliporphine, predicentrine, *N*-methylaurotetanine and corunine as well as the above two compounds. Injury also stimulated synthesis of oxoaporphine related and other polymeric pigments. Corunine was responsible for at least part of the color of discolored sapwood. None of the above compounds except glaucine was detected in normal sapwood or heartwood of *L. tulipifera*. Thus, formation of alkaloids and lignans in discolored sapwood differs both quantitatively and qualitatively from that observed during the normal transition of sapwood to heartwood in this tree. The compounds formed in response to injury differed substantially from one zone of injury to another within the same tree.

INTRODUCTION

When living trees of *Liriodendron tulipifera* are injured, the wood frequently shows spectacular changes in color ranging from dark yellowish-green, to pink, red, purple, blue, brown and even black. Thus, discolored wood contrasts markedly with the light yellowish green of normal heartwood and the light cream color of normal sapwood. Some of the remarkable chromophores formed in wounded trees are believed to be phytoalexins which contribute to the resistance of living trees to diseases caused by wood-destroying and other injurious microorganisms [1].

In a previous paper [2], we showed that normal sapwood of *L. tulipifera* contained a small amount of a single nonphenolic alkaloid—glaucine. By contrast, the normal heartwood of this species contained substantial amounts of six nonphenolic and two phenolic alkaloids, two lignans and one simple phenol. In addition, mass spectrographic evidence was obtained for the occurrence of 1-benzyltetrahydroisoquinoline alkaloids. Pathways for the biosynthesis of the above alkaloids and lignans from a common precursor—phenylalanine—were postulated in accordance with the suggestions of Gottlieb [3].

The purpose of the present investigation was to isolate, characterize, and identify certain of the major extraneous substances present in discolored sapwood formed in response to injury of *L. tulipifera*. A secondary purpose was to compare and contrast the compounds detected with those reported earlier in normal sapwood and normal heartwood of this important tree.

RESULTS AND DISCUSSION

Compounds isolated

Discolored sapwood of *L. tulipifera* is highly hegerogenous both in terms of color (Fig. 1a and b) and in terms of the types of amounts of compounds formed in response to injury of the tree stem (Table 1). The total amount and composition of the acidic methanol extractives varied with the particular zone of discolored sapwood tested (Table 2). The amount of extractives increased progressively with increasing coloration of the tissue when the tree stem was fresh cut.

A total of 19 alkaloids and lignans were isolated from the three zones of discolored sapwood tested (see Formulae 1). These compounds included: nine nonphenolic aporphine alkaloids—nornuciferine (**1**) [4] and its *N*-acetyl derivative (**2**), nuciferine (**5**) [5], norushinsunine (**6**), dehydroglaucine (**7**), norglaucine (**8**) [6], glaucine (**9**), liriodenine (**15**), and *O*-methylatheroline (**16**) [2], eight

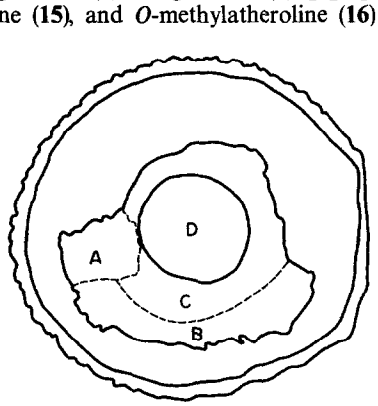


Fig. 1a.

*This paper is dedicated to Prof. Dr. K. Freudenberg on the occasion of his 90th birthday.

Table 1. Aporphine alkaloids and lignans isolated from normal sapwood, normal heartwood and discolored sapwood of *Liriodendron tulipifera*

Compound	Amount isolated (g/kg)*				
	Normal† sapwood	Normal† heartwood	Discolored sapwood		
			Zone A	Zone B	Zone C
Nonphenolic aporphine alkaloids					
Nornuciferine (**1**)		+	+	0.120	0.160
N-acetylnornuciferine (**2**)		0.270	0.120	++	++
Nuciferine (**5**)		+	+	0.030	+
Norushinsunine (**6**)		0.155	+	+	0.038
Dehydroglaucine (**7**)		0.078	+	+	+
Norglaucine (**8**)				0.130	+
Glaucine (**9**)	+	2.85	1.40	6.40	7.50
Liriodenine (**15**)		0.660	0.014	0.034	+
O-methylatheroline (**16**)		0.086	+	0.018	+
Phenolic aporphine alkaloids					
Asimilobine (**3**)		0.036	0.056	+	+
N-acetylasimilobine (**4**)		0.130	0.046	+	+
Thaliporphine (**10**)				+	0.058
Predicentrine (**11**)			0.162	0.247	0.280
N-methyllaurotetanine (**12**)			0.032	+	0.033
Lirioferine (**13**)			0.620	0.154	0.260
Liriotulipiferine (**14**)			0.407	0.203	0.750
Coruine (**17**)			+	0.006	0.008
Lignans					
Syringaresinol (**19**)		0.036	+	+	+
Syringaresinol, dimethyl ether (**20**)		0.860	0.210	++	++

* Amount of compound (g) isolated per kg air dry weight of wood. The signs + and + + indicate trace and substantial amounts detected by TLC, respectively, but were not quantified. † See ref. [2].

phenolic aporphine alkaloids—asimilobine (**3**) and its *N*-acetyl derivative (**4**), thaliporphine (**10**) [7], predicentrine (**11**) [8,9], *N*-methyllaurotetanine (**12**) [10], (+)-lirioferine (**13**), (+)-liriotulipiferine (**14**), and corunine (**17**) [11,12]; and two lignans—(+)-syringaresinol (**19**) and its dimethyl ether (**20**). The green alkaloid, corunine, accounts for at least part of the greenish color of the discolored sapwood.

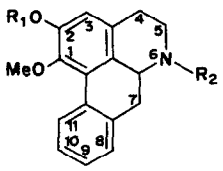
This is the first report of the natural occurrence of two of the sixteen compounds detected—(+)-lirioferine and (+)-liriotulipiferine. (+)-Lirioferine is the last of the four possible monophenolic 1,2,9,10-tetraoxygenated aporphine alkaloids to be found in nature; all four have now been shown to occur in *L. tulipifera*. Cassarmine, the *N*-methyl quarternary salt of (+)-lirioferine, was isolated earlier from roots of *Cocculus sarmentosus* [13]. Also, the *dl*-base of (+)-lirioferine has been synthesized [14,15].

Synthesis of glaucine was greatly stimulated by injury of *L. tulipifera*; it was the most abundant compound isolated from all three zones of discolored sapwood (Table 1). The amount isolated varied five-fold—from a minimum of 0.14% in zone A to a maximum of 0.75% by weight in zone C. Also, abundant in zone A were predicentrine, lirioferine, liriotulipiferine, and syringaresinol dimethyl ether. Glaucine and predicentrine were abundant in zone B. Glaucine predicentrine, lirioferine, and liriotulipiferine were abundant in zone C (Table 1).

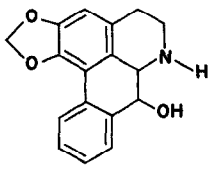
Table 2. Composition of acidic methanol extractives from normal heartwood and discolored sapwood of *Liriodendron tulipifera*

Fraction	Normal† heartwood	Discolored sapwood		
		Zone A	Zone B	Zone C
		(g/kg dry weight of tissues)		
Neutral substances, phenols, acids	6.5	4.7	4.0	4.5
Precipitate (mainly polymeric materials)	10.8	9.4	29.0	41.0
Violet pigments*	0.0	1.1	3.2	3.5
Dark blue polymeric pigments*	0.0	1.6	3.8	4.9
Nonphenolic alkaloids	8.9	4.1	7.8	9.3
Phenolic alkaloids	1.7	3.3	1.9	3.8
Green Pigments			0.4	0.6
Total 1% HCl–MeOH extract	27.9	24.2	50.1	67.6

* These fractions are currently being characterized. † See ref. [2].



(1) R_1 = Me; R_2 = H
(2) R_1 = Me; R_2 = Ac
(3) R_1 = R_2 = H
(4) R_1 = H; R_2 = Ac
(5) R_1 = R_2 = Me



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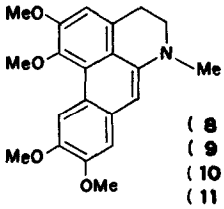

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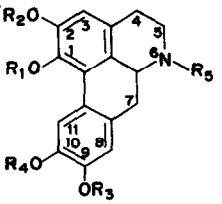
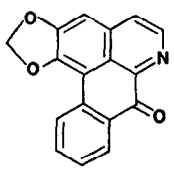


(8) R_1 = R_2 = R_3 = R_4 = Me; R_5 = H
(9) R_1 = R_2 = R_3 = R_4 = R_5 = Me
(10) R_1 = H; R_2 = R_3 = R_4 = R_5 = Me
(11) R_2 = H; R_1 = R_3 = R_4 = R_5 = Me
(11a) R_2 = Ac; R_1 = R_3 = R_4 = R_5 = Me
(12) R_3 = H; R_1 = R_2 = R_4 = R_5 = Me
(13) R_4 = H; R_1 = R_2 = R_3 = R_5 = Me
(13a) R_4 = Ac; R_1 = R_2 = R_3 = R_5 = Me
(14) R_2 = R_4 = H; R_1 = R_3 = R_5 = Me
(14a) R_2 = R_4 = Ac; R_1 = R_3 = R_5 = Me

Injury-induced synthesis of compounds which occur in normal heartwood and of distinctive compounds which do not occur in normal heartwood or sapwood has been observed in only a few other species [16–20].



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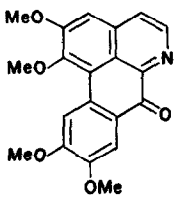

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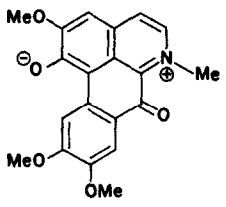

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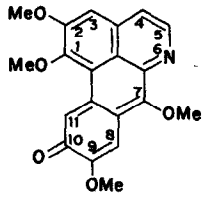
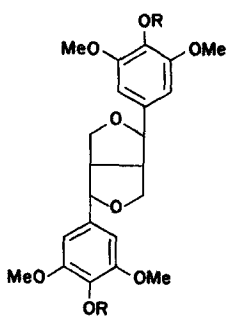


(18)



(19) R = H
(20) R = Me

Huang has recently shown that glaucine has antimicrobial activities against various wood-inhabiting fungi [21]. Thus, the greatly increased synthesis of glaucine demonstrated in this investigation may be a mechanism by which living trees of *L. tulipifera* protect themselves against attack by wood-destroying fungi that invade the tree stem following injury [1].

Comparison with normal tissues

Discolored sapwood differed markedly from both normal sapwood and normal heartwood of *L. tulipiferine.* It contained a substantially larger amount of acidic methanol extractives than normal sapwood (Table 2). Although normal sapwood contained only glaucine in trace amount, the broad array of compounds discussed below were found in discolored sapwood.

The three zones of discolored sapwood contained from slightly less to about 2.5 times as much acidic methanol extractives as did the normal heartwood of *L. tulipifera* (Table 2). The compounds present in discolored sapwood and normal heartwood also differed markedly both quantitatively and qualitatively. Although nonphenolic aporphine alkaloids predominated in normal heartwood, with the exception of glaucine, phenolic aporphines predominated in discolored sapwood. Six of the phenolic alkaloids isolated from discolored sapwood were not found, even in trace amounts, in normal heartwood. By contrast no new nonphenolic alkaloids were isolated from any of the samples of discolored sapwood tested. Lignans were detected in all three zones of discolored sapwood tested.

In addition to the specific compounds isolated and characterized as described below, the acidic methanol extracts of each zone of discolored sapwood contained about 0.11–0.35% by weight of mixture of violet oxoaporphine related pigments and about 0.16–0.49% of dark blue polymeric pigments. Both of these fractions are currently being characterized. Neither of these two types of pigments were found in normal sapwood nor normal heartwood. The extracts from both normal heartwood and discolored sapwood also contained 0.94–4.1% by weight of hot-water precipitable material that consisted mainly of unknown polymeric materials. The chemical nature of these substances is not being determined.

Identification and structural determination of the compounds isolated

Of the nineteen compounds isolated, ten were identified by mmp or direct comparison of their spectral and TLC data with the same compounds isolated and identified earlier from heartwood [2]. Six of the nine remaining compounds were identified by comparison of their spectral data with those reported by other investigators (see Experimental). Thus, structural determinations are discussed only for the three compounds which were not reported previously.

(+)-Lirioferine (**13**), $C_{20}H_{23}NO_4$ (M^+ *m/e* 341), mp 173–174°, $[\alpha]_D$ + 123.6, had UV, NMR and MS characteristic of 1,2,9,10-tetraoxygenated aporphines [22,23]. The compound gave a monoacetate and a methiodide. The bathochromic shift observed in the UV spectrum on addition of base showed the phenolic nature of the compound. The NMR spectrum indicated the presence of adjacent C-1 and C-2 methoxyl groups. This is in agreement with downfield shifts observed for H-8 and

H-11 ($\Delta\tau$ − 0.11 for both) upon acetylation. Consequently, the hydroxyl group is attached either at C-9 or C-10 [24]. The UV spectrum of the compound in methanol was similar to those of *N*-methyllaurotetanine (**12**) [25], and (±)-10-hydroxy-1,2,9-trimethoxyaporphine (**13**) [14]. In alkaline media, however, the compound gave a UV spectrum identical to that of the latter compound [14]. Therefore, (+)-lirioferine is (+)-10-hydroxy-1,2,9-trimethoxyaporphine (**13**) (see Formulae 1).

(+)-Liriotulipiferine (**14**), $C_{19}H_{21}NO_4$ (M^+ *m/e* 327), mp 184–186°, $[\alpha]_D$ +174.4, had UV, NMR and mass spectra characteristic of 1,2,9,10-tetraoxygenated aporphines [22, 23]. The compound gave a diacetate and a methiodide after treatment with acetic anhydride and methyl iodide, respectively. The phenolic nature of the compound was indicated by the bathochromic shift observed in the UV spectrum on addition of base. The NMR spectrum showed the presence of a C-1 methoxyl group. Furthermore, the shielding of these hydrogens ($\Delta\tau$ + 0.08) in comparison to that of the C-1 methoxyl group adjacent to the C-2 methoxyl group indicates the presence of a C-2 hydroxyl group (see Experimental). Upon acetylation, the chemical shifts for H-3 and H-11 showed characteristic downfield-shifts ($\Delta\tau$ − 0.13 and −0.15) indicating the presence of C-2 and C-10 hydroxyl groups [24]. Therefore, the structure **14**, 2,10-dihydroxy-1,9-dimethoxyaporphine, for (+)-liriotulipiferine is apparent (see Formalae 1).

Corunine (**17**), $C_{20}H_{17}NO_5$ (M^+, *m/e* 351), mp 245–247°. Dr. Castedo very kindly compared the green alkaloid which we isolated from *L. tulipifera* with known samples of corunine which Rikas, Sueiras and Castedo [11] had characterized after isolation from *Glaumium flavum* cv. *Vestium*. Structure **17** was established for the known samples by chemical synthesis [11, 12]. By mixed melting point, TLC, and spectral analysis Dr. Castedo established that our green alkaloid and his known samples were identical. We also found that the UV and NMR spectra of our alkaloid-corresponded to those reported for glauvine which Yakhomtova *et al.* [26] isolated from *Glaucium flavum*. These authors proposed structure **18** for their compound on the basis of its conversion into 10-hydroxy-1,2,9-trimethoxy-*N*-noraporphine upon reduction with zinc in HCl. But this proposed structure fails to explain: (a) the fact that the UV-spectrum of the compound isolated by Yakhontova *et al.* was similar to those of *O*-methylantheroline (**16**) in both conc. and dilute sulfuric acid; and (b) the fact that the three-proton singlet assigned by Yakhontova *et al* [26] to the C-7 methoxyl hydrogens was more substantially deshielded than those of the singlets for the C-1, C-2 and C-9 methoxyl groups (τ5.07 compared to 5.90, 5.86 and 5.66, respectively). After repeating both of these tests with our green alkaloid, we conclude that glauvine as reported by Yakhontova *et al.* [26] and corunine as reported by Ribas *et al.* [11] have the same structure. Both we and Dr. Castedo also concluded that structure **17** is correct for the compounds isolated by both of these other authors and for our green alkaloid [27].

NMR characteristics of the 1,2,9,10-*tetraoxygenated aporphines*

The NMR spectral data for the aporphine alkaloids and their acetates are given in Table 3. The C-1 methoxyl hydrogens adjacent to a C-2 hydroxyl group are more shielded ($\Delta\tau$ *ca* +0.08) and H-3 more deshielded ($\Delta\tau$ *ca* −0.05) than the corresponding hydrogens adjacent to C-2 methoxyl group. Acetylation of phenolic hydroxyl groups generally produces deshielding ($\Delta\tau$ −0.11 ~ −0.15) of hydrogens *ortho* to the hydroxyl group. In the case of C-10 hydroxyl derivatives, however, the hydrogens *meta* to the hydroxyl group (H-8) also showed a deshielding effect ($\Delta\tau$ ~ −0.10).

Related work on the alkaloids and lignans of normal heartwood of *L. tulipifera* has been published by Hufford and co-workers [28–30].

EXPERIMENTAL

Mp's are uncorrected. Preparative YLC was carried out with Si gel 60 F-254 (Merck) plates. Me_2CO was used to

Table 3. NMR characteristics* of aporphine alkaloids isolated from discolored sapwood of *Liriodendron tulipifera*

Compounds	NMe	Methoxyl C-1	Methoxyl C-2	Methoxyl C-9	Methoxyl C-10	H-3	H-8	H-11	Other
Norglaucine (**8**)		6.36	6.14	6.14	6.14	3.40	3.24	1.89	
Glaucine (**9**)	7.50	6.40	6.17†	6.15†	6.12†	3.43	3.23	1.88	
Thaliporphine (**10**)	7.50		6.16	6.16	6.16	3.46	3.23	1.90	
Predicentrine (**11**)	7.49	6.48		6.16†	6.14†	3.37	3.21	2.02	
Acetate of **11** (**11a**)	7.50	6.48		6.16†	6.14†	3.24	3.21	1.97	7.72 (C-2 COMe)
N-Methyllaurotetanine (**12**)	7.52	6.41	6.16		6.16	3.42	3.20	1.90	
Lirioferine (**13**)	7.53	6.40	6.20†	6.16†		3.43	3.26	1.97	
Acetate of **13**(**13a**)	7.52	6.42	6.20	6.20		3.42	3.15	1.86	7.76 (C-10 COMe)
Liriotulipiferine (**14**)	7.43	6.48		6.14		3.38	3.24	2.06	3.96 (*br* C-2 and C-10 OH)
Acetate of **14** (**14a**)	7.54	6.50		6.19		3.25	3.14	1.91	7.79 and 7.75 (C-2 and C-10 COMe)
Asimilobine (**3**)		6.48				3.30		1.66	2.72 (*ABC m*: H-8, H-9 and H-10)
Corunine (**17**)‡	5.15		5.98†	5.92†	5.88†				
17 in CF_3COOD	5.07		5.90†	5.86†	5.66†	2.40	1.94	0.97	1.54 and 1.34 (both *d*, J_{AB} 6.4 Hz: H-4 and H-5)

* All in $CDCl_3$ unless specified otherwise; chemical shifts are expressed in τ-values with TMS as an internal reference. † These values may be interchanged. ‡ These signals in the aromatic region were too weak to be analyzed because of the low solubility of the compound.

recover substances from the Si gel. The 3 samples of discolored sapwood used in this investigation were obtained from a 45-yr-old tree harvested near Zebulon, N.C. The tree contained an inner core of 12 annual increments of apparently normal heartwood and 13 annual increments of discolored sapwood, surrounded by an outer cylinder of 20 annual increments of apparently normal sapwood (Fig. 1). The tree had been injured (apparently by fire) at least 2×—at 12 and 25 yr of age. The discolored sapwood formed after the first (innermost) injury extended completely around the stem while that formed after the second (outermost) injury extended around 40% of the circumference. Cross-sectional discs were cut from the stem in sequence and each disc then split parallel with the grain to give the following three zones. Zone A extended from annual rings 12 to 25 counting from the pith; it included about 10% of the circumference of the stem at ages 12 and 25. Zone C extended from annual rings 12 to 18 and Zone B from rings 18 to 25 counting from the pith; both zones included about 30% of the circumference. Zone A was a fairly homogenous greenish–brown color when fresh cut whereas Zones B and C were greenish–brown with blotches of darker purple color (Fig. 1). The regions of purple tissue were larger in extent within Zone C than within Zone B. Thus, Zones A–C formed a series of progressively darker coloration. All 3 zones became progressively darker in color during the first 48 hr after cutting the stem. Tissue from each zone was air-dried, ground to pass a 40-mesh screen, preextracted with ligroin to remove waxes and then air-dried again. 1 kg tissue from each zone was then steeped in 6 l. 1% HCl–MeOH at room temp. for 24 hr and then filtered. Wet wood meal was then treated again with 4 l. 1% HCl–MeOH as before. Combined extracts were concd to about 200 ml and then added slowly to 800 ml Et_2O. The Et_2O soln obtained from Zones B and C were decanted off, washed with H_2O, dried, and the solvent evaporated to obtain a *mixture of neutral substances, phenols and acids.* The brown ether-insoluble mass obtained from all 3 zones were stirred for 30 min with 500 ml hot H_2O. The water-insoluble ppt. was filtered off, washed 2× with 150 ml hot H_2O and then discarded. The filtrate and washing were combined, and washed 2× with 20 ml Et_2O and then adjusted to pH 2 (pH 1.5 in the case of extracts from Zones B and C) by adding conc. NH_4OH dropwise. The soln was then extracted continuously with 500 ml Et_2O (1000 ml in the case of Zones B and C). The Et_2O soln was washed 2× with 100 ml H_2O, dried, and the solvent evaporated to give a *mixture of violet pigments.* The aq layer was then adjusted to pH 7.5–8.0 with conc. NH_4OH. The purple ppt. (dark blue in the case of Zones B and C) was then filtered off, washed with H_2O, and dried to give a *mixture of dark blue polymeric pigments.* Filtrate from Zone A was then shaken with $CHCl_3$ (200 ml × 4). The $CHCl_3$ soln was washed with H_2O, dried, and solvent evaporated to give a *total alkaloid mixture.* This mixture was dissolved in 400 ml Et_2O shaken 4× with 100 ml 0.5 N NaOH, washed with H_2O, dried, and solvent evaporated to give a *mixture of nonphenolic alkaloids.* Filtrates from Zones B and C from which the mixture of polymeric pigments were removed were extracted continuously with Et_2O for 48 hr, washed with H_2O, concd to about 400 ml and then shaken 3× with 100 ml 0.5 N NaOH. The Et_2O layer was then similarly washed with H_2O, dried, and the solvent evaporated to give a *mixture of nonphenolic alkaloids.* The alkaline soln was adjusted to pH 7.8–8.0 with 2N HCl, shaken 3× with 100 ml $CHCl_3$ (150 ml in the case of Zones B and C). The $CHCl_3$ solns were then washed with H_2O, dried, and solvent evaporated to give a *mixture of phenolic alkaloids.* Combined ether solns from Zone A (neutral and acidic materials) were concd to about 300 ml, shaken 3× with 150 ml 0.5 N $NaHCO_4$, 4× with 50 ml 0.5 N NaOH, washed with H_2O, dried and solvent evaporated to give a *mixture of neutral substances.* Both alkaline solns from Zone A were neutralized and extracted 3× with 100 ml $CHCl_3$. These $CHCl_3$ solns were washed with H_2O, dried, and solvent evaporated to give a *mixture of phenols* and a *mixture of acids.* In the case of extracts from Zones B and C, the aq. layer from which the total alkaloids were removed was extracted 3× with 100 ml $CHCl_3$. The $CHCl_3$ soln was washed with H_2O, dried, and the solvent evaporated to obtain a *mixture of green pigments.* The amounts of each of the above fractions isolated from normal heartwood [2] and the three zones of discolored sapwood are given in Table 2.

The mixture of neutral substances from Zone A was fractionated by preparative TLC using $CHCl_3$–EtOAc (9:1) to isolate: N-*acetylnornuciferine* (**2**). The crude product obtained from the minor TLC band was recrystallized from C_6H_6–cyclohexane to give colorless needles (120 mg), mp 227–230°. The identity of the compound was established by mmp and comparison of IR spectral and TLC data with those for an authentic sample [2].

(+)-*Syringaresinol dimethyl ether* (**20**). The crude product obtained from the major TLC band was recrystallized from MeOH to give colorless plates (210 mg), mp 115–119°. The identity of the compound was established by mmp and comparison of IR spectral and TLC data with those for an authentic sample [2].

The mixture of phenols from Zone A was fractionated by preparative TLC using C_6H_6–MeOH (9:1) to isolate: N-*acetylasimilobine* (**4**). The crude product obtained from the major TLC band was recrystallized from $CHCl_3$ to give colorless needles (46 mg), mp 280–283°. The identity of the compound was established by mmp and comparison of IR spectral and TLC data with those for an authentic sample [2]. From the next TLC fraction, (+)-syringaresinol (**19**) was detected by TLC, but could not be isolated.

The mixture of acids from Zone A and the *mixture of neutral substances, phenols and acids from Zones B and C* were not fractionated or characterized.

The mixture of violet pigments and the *dark blue polymeric pigments* in all 3 zones of discolored sapwood are currently being fractionated and characterized. Violet pigments were shown to include oxoaporphine related substances on the basis of their UV spectra in H_2SO_4.

The mixture of nonphenolic alkaloids from Zone A was fractionated by preparative TLC using cyclohexane-EtOAc–$NHEt_2$ (7:2:1) to isolate compounds **9** and **15**; the minor constituents of this mixture were not investigated.

Glaucine (**9**). The crude product obtained from the major TLC band was recrystallized from EtOAc to give colored needles (1.4 g), mp 119–121°. The identity of the compound was established by mmp and comparison of IR spectral and TLC data with those for an authentic sample [2].

Liriodenine (**15**). The crude product obtained from the last TLC band was recrystallized from $CHCl_3$ to give yellowish–green needles (14 mg), mp 276–280°. The identity of the compound was established by mmp and comparison of IR spectral and TLC data with those for an authentic sample [2].

The mixture of nonphenolic alkaloids from Zone B was dissolved in 30 ml hot EtOAc, and kept at 0° for several days. Crude crystals were filtered off, recrystallized from EtOAc to give *glaucine* (**9**, 3.5 g), colorless prisms, mp 117–119° [2]. After evaporation of solvent from the mother-liquor, the residue was chromatographed on preparative TLC plates using cyclohexane–EtOAc–$NHEt_2$ (7:2:1) to isolate the following compounds: *Nuciferine* (**5**). The brown oil recovered from the 1st band was dissolved in a small amount of MeOH, and kept at 0° for several days. Crude crystals were filtered off, recrystallized from MeOH to give colorless needles (30 mg), mp 135–137° (Lit. [5] 165.5°). NMR ($CDCl_3$): τ 7.48 (3H, *s*, NMe), 6.40 (3H, *s*, C-1 OMe), 6.16 (3H, *s*, C-2 OMe), 3.38 (1H, *s*, H-3), 2.74 (3H, *ABC m*, H-8, H-9 and H-10), 1.60 (1H, *d*, J_{AX} 8.0 Hz, H-11). The NMR spectrum was similar to that of nornuciferine (**1**) except it contains an additional N-Me signal. MS: *m/e* (rel. int.) 296 (9): 295 (M^+, 33), 294 (32), 280 (17), 264 (14), 252 (11), 43 (100).

Nornuciferine (**1**). The brown oil recovered from the 3rd band was dissolved in a small amount of 1% HCl–MeOH, and kept at 0° for 2 days. Crude crystals were filtered off,

recrystallized from MeOH to give *nornuciferine-hydrochloride*, colorless needles (120 mg), mp 245–246° UV: λ_{max} in MeOH 310 (*sh*), 270 (*sh*), 268, 265 (*sh*) and 230 nm. The UV spectrum corresponded to that of the (±)-base-hydrochloride reported by Weisbach and coworkers [31]. The free base obtained from the *hydrochloride* would not crystallize but was homogenous on TLC and NMR. NMR ($CDCl_3$): τ 6.39 (3H, *s*, C-1 OMe), 6.17 (3H, *s*, C-2 OMe), 3.38 (1H, *s*, H-3), 2.76 (3H, *ABC m*, H-8, H-9, and H-10), 1.60 (1H, *d*, J_{AX} 8.0 Hz, H-11). MS: *m/e* (rel. int.) 282 (13), 281 (M^+, 70), 280 (100), 279 (*m**), 266 (20), 252 (8), 251.8 (*m**), 251 (6), 250 (20), 222.4 (*m**).

Norglaucine (**8**). The brown oil recovered from the 4th band was dissolved in a small amount of 1% HCl–MeOH, and kept at 0° overnight. The crude crystals were filtered off, recrystallized from methanol to give *norglaucine-hydrochloride*, colorless needles (130 mg), mp 221–222°. The free base obtained from the *hydrochloride* would not crystallize but homogenous on TLC and NMR (see Table 3). The NMR is identical with the one reported by Johns and coworkers [6]. MS: *m/e* (rel. int.) 342 (18), 341 (M^+, 83), 340 (100), 339.0 (*m**), 326 (31), 324 (31), 311.7 (*m**), 310 (23), 298 (12), 280.8 (*m**).

Glaucine (**9**, 2.9 g), *O*-methylatheroline (**16**, 18 mg), yellowish-orange needles, mp 230–230° [2], and *liriodenine* (**15**, 34 mg), yellowish-green needles, mp 278–281° [2], were obtained from the 2nd, 5th and 6th bands respectively. The total yield of *glaucine* (**9**) was 6.4 g.

The mixture of nonphenolic alkaloids from Zone C was dissolved in 50 ml hot EtOAc, and kept at 0° for several days. Crude crystals were filtered off, recrystallized from EtOAc to give *glaucine* (**9**, 4.3 g), colorless prisms, mp 117–119° [2]. After evaporation of EtOAc from the mother-liquor, residue was dissolved in 100 ml 1% HCl–MeOH, and kept at 0° overnight. Crude crystals were filtered off, recrystallized from MeOH to give *glaucine-hydrochloride* (0.4 g), colorless needles, mp 234–235°. The mother-liquor was then concentrated to *ca.* 30 ml, diluted with 30 ml water, and adjusted to pH 7.5–8 with 1N NH_4OH. The soln was extracted continuously with 300 ml Et_2O for 48 hr. The Et_2O soln was washed with H_2O, dried and solvent evaporated to recover a *mixture of nonphenolic alkaloids* (3.6 g) which was then chromatographed on preparative TLC plates using cyclohexane–EtOAc–$NHEt_2$ (7:2:1) to isolate the following compounds: *glaucine* (**9**, 3.0 g), nornuciferine (**1**) isolated as *hydrochloride* (160 mg), colorless needles, mp 245–246°, and norushinsunine (**6**, 38 mg), colorless needles, mp 200–203° [2], were obtained from the 2nd, 3rd and 4th bands respectively. The total yield of *glaucine* (**9**) was 7.5 g. *Nuciferine* (**5**), *norglaucine* (**8**), *liriodenine* (**15**) and *O-methylatheroline* (**16**) were detected in the fractions obtained from the 1st, 5th and 6th TLC bands, respectively.

The mixture of phenolic alkaloids from Zone A of discolored sapwood was fractionated by preparative TLC using the above solvent to isolate the following compounds: (+)-*Lirioferine* (**13**). The crude product obtained from the 1st TLC band was recrystallized from EtOAc to give colorless needles (620 mg) mp 173–174°, $[\alpha]_D$ in $CHCl_3$, +128.6 UV: λ_{max} in MeOH, 312 (*sh*), 303, 280, 273 (*sh*) and 220 nm (4.02, 4.07, 4.09, 4.02 and 4.50); λ_{max} in 0.05 N MeOH–MeONa, 330, 305, 286 (*sh*), 281, 256 (*sh*), 251 and 230 nm (3.85, 3.74, 3.92, 3.93, 4.25, 4.26, 4.48). The UV spectra were identical with those of the (±)-base reported by Baarshers Arndt [14]. MS: *m/e* (rel. int.), 342 (24), 341 (M^+, 100), 340 (98), 339 (*m**), 327 (11), 326 (45), 311.7 (*m**), 311 (10), 310 (29), 298 (18), 296 (6), 295 (9), 283 (14), 280.7 (*m**), 268.8 (*m**), 268 (7), 267 (20), 260.4 (*m**), 239.2 (*m**). Methiodide: colorless scales, mp 257–259° (dec.) Monoacetate: colorless gum, M^+ *m/e* 383 (100). NMR (see Table 3).

Predicentrine (**11**). The brown oil recovered from the 2nd TLC band was treated with 1% HCl–MeOH. Crude crystals of the hydrochloride were collected and recrystallized from MeOH to give colorless needles (162 mg), mp 208–214° (dec.). The base obtained by neutralization of the hydrochloride could not be crystallized, but was homogenous on TLC and NMR (see Table 3). UV: λ_{max} in MeOH, 312 (*sh*), 302, 281, 274 (*sh*) and 217 nm (4.03, 4.11, 4.14, 4.04 and 4.55); λ_{max} in 0.05 N methanolic MeONa, 325 (*sh*), 303, 297 (*sh*), 282 and 246 nm (3.65, 4.06, 4.04, 4.09 and 4.25). MS: *m/e* (rel. int.), 342 (27), 341 (M^+, 100), 340 (83), 339 (*m**), 327 (14), 326 (51), 311.7 (*m**), 311 (9), 310 (28), 298 (19), 294 (7), 283 (15), 280.7 (*m**), 268.8 (*m**), 268 (7), 267 (9), 260.4 (*m**). Monoacetate: colorless gum, M^+, *m/e* 383 (100), NMR (see Table 3). NMR spectra for the free base and the monoacetate were identical with those reported by Johns *et al.* [8].

Asimilobine (**3**). The brown oil recovered from the 3rd TLC band was rechromatographed on Si gel plate to isolate **3**. The product (56 mg) could not be crystallized. NMR and MS spectra of the product indicated that is contained a small amount of **12** as an impurity. See Table 3 for NMR data. M^+, *m/e* 267. The NMR was identical to that of authentic sample [2].

N-*Methyllaurotetanine* (**12**). Crude product (32 mg) was obtained from the rechromatography of the 3rd TLC band in addition to **3**. The product could not be crystallized. NMR and MS spectra of the product indicated that it contained a small amount of **3** as an impurity. See Table 3 for NMR data. MS: *m/e* (rel. int.), 342 (23), 341 (M^+, 100), 340 (94), 327 (15), 326 (54), 311 (8), 310 (24), 299 (6), 298 (23), 283 (17).

(+)-*Liriotulipiferine* (**14**). The crude product obtained from the 4th TLC band was treated with 1% MeOH–HCl. The crude crystals of the hydrochloride were collected and recrystallized from MeOH to give colorless needles (407 mg), mp 218–220° (dec.). The base obtained by neutralization of the hydrochloride was recrystallized from EtOAc to give colorless needles, mp 184–186° $[\alpha]_D$ in CCl_4, +174.4. UV: λ_{max} in MeOH, 313 (*sh*), 303, 281, 274 (*sh*) and 218 nm (log ε 4.03, 4.10, 4.12, 4.06 and 4.54); λ_{max} in 0.05 N MeOH–MeONa, 320, 312 (*sh*), 282 and 248 nm (log ε 3.91, 3.90, 3.96 and 4.40). MS: *m/e* (rel. int.), 328 (26), 327 (M^+, 100), 326 (99), 313 (12), 312 (52), 311 (28), 297 (12), 296 (36), 284 (28), 269 (24). Methiodide: colorless scales, mp 214–216 (dec.). Diacetate: colorless gum, M^+, *m/e* 411 (100). NMR (see Table 3). The mother-liquor from the recrystallization of the hydrochloride was neutralized and the basic fraction recovered was subjected to MS-examination. An intense ion peak was observed at *m/e* 192 with less intense ion peaks at *m/e* 177, 327 and 326.

The mixture of phenolic alkaloids from Zone B was chromatographed on preparative TLC plates in the same manner described for the corresponding mixture obtained from Zone A to isolate the following compounds: (+)-*lirioferine* (**13**, 154 mg), colorless prisms, mp 181–183°, *predicentrine* (**11**, 247 mg), and (+)-*liriotulipiferine* (**14**, 203 mg), colorless prisms, mp 179–182°, were obtained from the 2nd, 3rd and 4th bands respectively. *Prediecentrine* was purified as the *hydrochloride* as described above. *Thaliporphine* (**10**) was detected in the mother-liquor obtained from the recrystallization of **13** by TLC.

The mixture of phenolic alkaloids from Zone C was chromatographed on preparative TLC plates in the same manner described for the corresponding mixture obtained from Zone A to isolate the following compounds: *Thaliporphine* (**10**). The crude product obtained from the 1st band was recrystallized from EtOAc to give colorless needles (58 mg), mp 168–170° (Lit. [7] 170–172°). UV: λ_{max} in MeOH, 307 (*sh*), 303, 279, 272 (*sh*) and 220 nm (log ε 4.09, 4.11, 4.08, 4.00 and 4.50); λ_{max} in 0.05 N MeOH–NaOMe, 303, 298, 275, 267 (*sh*) and 216 nm (log ε 3.79, 3.81, 3.96, 3.93 and 4.39). The UV and NMR spectra (see Table 3) corresponded to those reported by Shamma and coworkers [7,25]. MS: *m/e* (rel. *int.*), 342 (23), 341 (M^+, 100), 340 (82), 339 (*m**), 327 (8), 326 (30), 325 (8), 324 (23), 311.7 (*m**), 311 (5), 310 (12), 309 (13), 299 (6), 298 (29), 297 (8), 283 (8), 281 (6), 280.7 (*m**), 280 (5), 279 (8), 268.8 (*m**), 268 (8), 267 (26), 266 (9), 260.4 (*m**), 260 (10), 239.2 (*m**).

(+)-*Lirioferine* (**13**, 260 mg). Colorless prisms, mp 172–174°, *precidentrine* (**11**, 280 mg), impure N-*methyllaurotetanine* (**12**, 33 mg), and (+)-liriotulipiferine, (**14**, 750 mg), colorless prisms, mp 182–184° were obtained from the 2nd, 3rd, 4th and 5th bands respectively. *Predicentrine* was purified as the *hydrochlo-*

ride, colorless needles, mp 212–215°; the free base would not crystallize but was homogenous on TLC and NMR.

The mixture of green pigments from Zones B and C was chromatographed on preparative TLC plates using $CHCl_3$–EtOAc (9:1) to isolate *corunine* (**17**). The dark green solid recovered from the last band was recrystallized from methanol to give dark green needles (6 mg from Zone B and 8 mg from Zone C), mp 245–247°. The identity of the compound was established by mp and comparison of UV, IR spectral and TLC data with those for an authentic sample (mp 254–255°) [27] UV: λ_{max} in MeOH or 0.05 N MeOH–MeONa 614 (*sh*), 600, 420 (*sh*, *br*), 392, 320 and 257 nm (log ε 3.63, 3.64, 3.83, 3.91, 4.57 and 4.45); λ_{max} in 0.05 N HCl–MeOH 490 (*sh*), 378, 286 and 254 nm (log ε 3.64, 4.11, 4.40 and 4.54); λ_{max} in conc. H_2SO_4 527, 445, 375, 360 (*sh*), 290 (*sh*) and 249 nm (log ε 4.13, 4.10, 3.74, 3.64, 4.23 and 4.54). M^+, *m/e* 251 The NMR (see Table 3) were identical with those reported by Yakhontova *et al.* [26].

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