A New 5-Formylbilinone as the Major Chlorophyll *a* Catabolite in Tree Senescent Leaves

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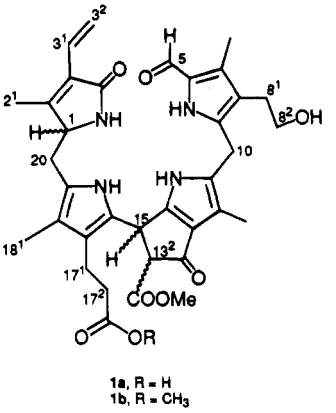
Senescence is an integral part of leaf development.[1] Occurring as a consequence of either age-related biological processes or induced processes, it is intimately linked to chlorophyll degradation.[2] We had shown that chlorophyll biosynthesis is arrested before the onset of senescence.[3] The interruption of chlorophyll biosynthesis in the leaf therefore precedes its degradation. Until very recently, the latter was very difficult to pinpoint at a molecular level. The first unambiguous identification of chlorophyll *a* catabolites was reported in 1991;[4,5] identification of other catabolites soon followed[6-8] (Scheme 1).

To establish if a chlorophyll catabolic pathway analogous to those described above is operative in leaves of a major plant under natural age-controlled senescent conditions, we examined the yellowing autumn leaves of *Liquidambar styraciflua* (sweet gum), a species of deciduous trees of the witch hazel family (*Hamamelidaceae*) widely distributed over the Americas, ranging from southern Illinois to central Argentina. Several fossil species of *Liquidambar* are known. The autumn leaves of *L. styraciflua* were picked from Illinois trees during September–October and from Argentine trees during March–April. They both showed the same pattern of chlorophyll catabolism. The freshly picked yellowing leaves (270 g) were extracted with acetone:methanol (3:1, v/v), the extracts were concentrated and diluted with water, the solution was adjusted to pH = 5.0, and the pigments were extracted into methylene chloride. They were then purified by TLC on silica gel using methylene chloride:acetone:methanol (8:1:1) as developer. A main colorless band (R_f 0.50; spotted by UV_{250} light) increased as the chlorophyll content of the leaves decreased due to the natural senescence process (yellowing or reddening of leaves). The substance was isolated (26 mg) by using preparative TLC plates on silica gel and acetone:methanol (95:5) as the developing solvent. It was crystallized from methylene chloride–hexane and was found to be



1a, R = H
1b, R = CH_3

Figure 1. Formylbilinone isolated from senescent leaves of *Liquidambar styraciflua* and *L. orientalis*.

the 5-formylbilinone **1a** (Figure 1). Its parent ion using a ZAB-SEQ spectrometer and FAB^+ ionization with Cs at 30 KeV in a glycerol/thioglycerol matrix gave M + H^+ = 645 appropriate for $C_{35}H_{40}N_4O_8$: UV–vis (nm) 210 (4.49), 235 (sh, 4.29), 307 (4.16); ^{1}H-NMR (300.13 MHz, $CDCl_3$:CD_3OD, 3:1, v/v, δ = 3.3 for HD_2COD) 9.22 (s, NH(21), 9.12 (s, H5), 6.37 (dd, J = 17.7, 11.5 Hz, H3^1), 6.07 (dd, J = 17.7, 2.2 Hz, H3$^2_{trans}$), 5.35 (dd, J = 11.5, 2.2 Hz, H3$^2_{cis}$), 4.88 (d, J = 4.3 Hz, H15, it is a singlet at 4.89), 3.87, 3.80 (AB_{sist}, J = 16.0 Hz, H10), 3.77 (d, J = 4.3 Hz, H13^2, exchangeable with D), 3.73 (s, $H_3$13^5), 3.69 (m, H1), 3.55 (m, H8^2), 2.81 (dd, J = 14.4, 4.4 Hz, H_B20), 2.70–2.55 (m, $H_2$8^1 and $H_2$17^1), 2.45–2.20 (m, H_A20 and $H_2$17^2), 2.14 (s, $H_3$7^1), 2.12 (s, $H_3$12^1), 1.98 (s, $H_3$2^1), 1.81 (s, $H_3$18^1); ^{13}C-NMR (75.465 MHz, $CDCl_3$) 190.0 (C13^1), 177.3 (C5), 176.6, 173.7, 170.4 (C4, C13^3, C17^3), 159.7, 155.1, 138.9, 136.1, 132.2, 128.1, 127.9, 125.3, 124.0, 123.4, 120.1, 119.0, 115.3, 112.4 (14 C_{arom}), 125.7 (C3^1), 119.4 (C3^2), 61.6 (C8^2), 60.6 (C1), 56.3 (C13^2), 52.7 (C13^5), 36.5 (C15), 35.8 (C17^2), 28.8 (C20), 26.9 (C8^1), 22.8 (C10), 20.1 (C17^1), 12.5 (C12^1), 9.0 (C2^1, C7^1, C18^1). The cleavage site of the macrocycle at C-5 has been established using the 1D NOE experiments previously described for model compounds,[5-7,9] where the connection H(5)-H_3(7^1) has been established and linked with the other side-chain substituents of the macrocycle. The methoxy group was assigned on the basis of its chemical shift and its unique position at the C(13^2) carboxylate in the chlorophyll.

Pigment **1a** (20 mg) was esterified to **1b** using BOP[10] (50 mg) in methanol:methylene chloride (2:1) in the presence of triethylamine during 3 h/20 °C. The methyl ester was purified on silica gel TLC using methylene chloride:acetone:methanol (80:14:6) and was crystallized from methylene chloride:hexane (18.6 mg, 91%): FAB-MS 659 (M + H^+) appropriate for $C_{36}H_{42}N_4O_8$; UV–vis (nm) 212 (4.42), 235 (sh, 4.27), 308 (4.18); ^{1}H NMR ($CDCl_3$:CD_3OD, 3:1) 9.16 (s, NH(21)), 9.10 (s, H5), 6.35 (dd, J = 17.7, 11.5 Hz, H3^1), 6.05 (dd, J = 17.7, 2.2 Hz, H3$^2_{trans}$), 5.35 (dd, J = 11.5, 2.2 Hz, H3$^2_{cis}$), 4.87 (d, J = 3.3 Hz, H15), 3.88, 3.84 (AB_{sist}, J = 16.0 Hz, H10), 3.78 (m, H1), 3.76 (s, $H_3$17^5), 3.73 (d, J = 4.3 Hz, H13^2, not exchanging with D as fast as in **1a**, very likely due to absence of the carboxylate at 17^3), 3.60 (s, $H_3$13^5), 3.60 (m, H8^2), 2.81 (dd, J = 14.6, 4.6 Hz, H_B20), 2.61 (m, $H_2$8^1,

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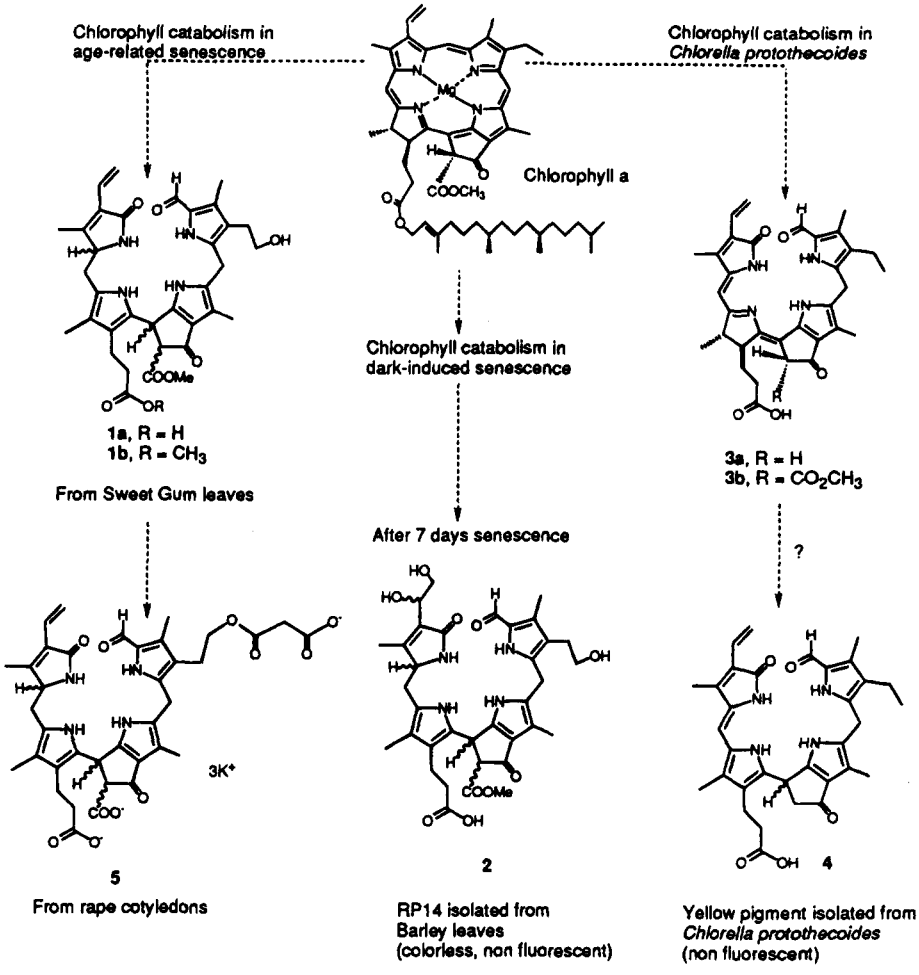
0022-3263/95/1960-6664$09.00/0

Scheme 1. Chlorophyll Catabolism in Age-Related and Dark-Induced Senescence and in Algae Cultures



$H_2 17^1$), 2.46–2.28 (m, $H_A 20$, $H_2 17^2$), 2.17 (s, $H_3 7^1$), 2.12 (s, $H_3 12^1$), 1.99 (s, $H_3 2^1$), 1.79 (s, $H_3 18^1$); ^{13}C-NMR ($CDCl_3$:CD_3OD, 3:1) 188.8 ($C13^1$), 175.9 (C5), 173.8, 173.6, 169.8 (C4, $C13^3$, $C17^3$), 158.5, 154.0, 139.2, 135.2, 132.8, 128.2, 128.1, 125.2, 123.8, 122.1, 120.1, 118.5, 115.2, 111.6 (14 C_{arom}), 125.4 ($C3^1$), 119.4 ($C3^2$), 67.2 ($C13^2$), 61.8 ($C8^2$), 60.0 (C1), 52.5 ($C13^5$), 51.6 ($C17^5$), 35.8 (C15), 35.6 ($C17^2$), 28.1 (C20), 27.0 ($C8^1$), 22.7 (C10), 19.8 ($C17^1$), 12.7 ($C12^1$), 9.1 ($C7^1$, $C18^1$), 8.8 ($C2^1$). The connectivities of the porphyrinate-bound hydrogens and the ^{13}C-atoms are supported by the 2D-$^1J_{HC}$ HETCOR correlation spectrum.

Autumn leaves of *L. orientalis* (one of the three Asiatic species of the genus *Liquidambar*) picked in southern Illinois also revealed that **1a** is the major catabolite of chlorophyll *a*.

Having established the structure of **1a** as the major detectable catabolite of chlorophyll *a* in natural senescent tree leaves, we can place it in the general outline of what is presently known about chlorophyll catabolism (Scheme 1). The formation of the conformationally more flexible open chain oligopyrroles[11] offer metabolic advantages. Bilanes are very flexible, and this will contribute to their transport and therefore to their remobilization within the nitrogen pool,[12] which is an essential feature of senescence.[13] It is noteworthy that in algae, which can rid themselves of the chlorophyll catabolites by excreting them into the medium, biladienes and bilenes (**3** and **4**) are isolated from the latter; reduction to bilanes does not seem so much at premium. The second metabolic advantage of bilanes such as **1**, **2**, or **5** is undoubtedly their susceptibility to degradation by electrophilic (oxidative) attack at the pyrrylmethane bridges.[14,15] While bilitrienes (such as biliverdins) or biladienes (such as **3** or bilirubin) cannot be degraded to monopyrroles, bilanes can, thus providing a rapid source for nitrogen reutilization. This last process takes place very likely in the soil, among the fallen leaves with a high content of **1a**.

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Supporting Information Available: 2D-$^1J_{HC}$ correlations and HETCOR spectrum of **1b** (1 page).

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