ORGANIC LETTERS

2009
Vol. 11, No. 14
3020−3023

Structure and Formation of the Fluorescent Compound of *Lignum nephriticum*

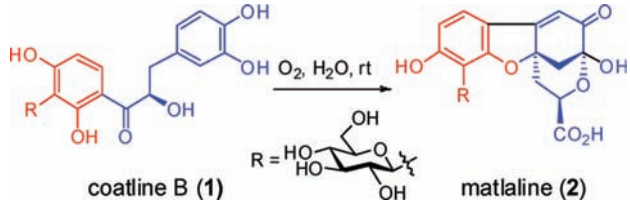
A. Ulises Acuña,*,† Francisco Amat-Guerri,*,‡ Purificación Morcillo,‡ Marta Liras,‡ and Benjamín Rodríguez‡

Instituto de Química-Física "Rocasolano" (IQFR), CSIC, Serrano 119, 28006 Madrid, Spain, and Instituto de Química Orgánica General (IQOG), CSIC, Juan de la Cierva 3, 28006 Madrid, Spain

roculises@iqfr.csic.es

Received May 11, 2009

ABSTRACT



The intense blue fluorescence of the infusion of *Lignum nephriticum* (*Eysenhardtia polystachya*), first observed in the sixteenth century, is due to a novel four-ring tetrahydromethanobenzofuro[2,3-*d*]oxacine which is not present in the plant but is the end product of an unusual, very efficient iterative spontaneous oxidation of at least one of the tree's flavonoids.

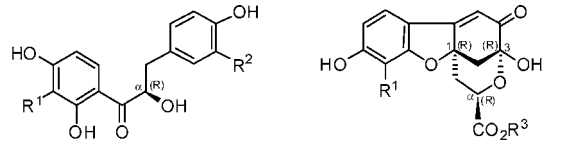
Fluorescence from a liquid solution was first reported in 1565 by Monardes[1] as the blue tinge of the infusion of a Mexican medicinal wood, known in Europe as *Lignum nephriticum*.[2,3] Throughout the centuries, Boyle, Newton, Herschel, and many other scientists were intrigued by this unusual phenomenon.[2] However, by the time at which fluorescence could be interpreted in electronic terms, the botanical origin of *L. nephriticum* was uncertain, and hence, the molecular structure of the earliest fluorophore remained unknown. From the arduous search of the source of *L. nephriticum*,[4−6] two genera of trees appeared as plausible candidates: *Eysenhardtia* Kunht and *Pterocarpus* Jacq. We found in sixteenth-century manuscripts of Sahagún[7] that Aztec healers had already noticed the "blue" color ("matlali" in Nahuatl) of the infusion of coatli, a plant used to treat urinary disorders.[2] These observations, together with more detailed accounts from Mexican botanists,[4] leave little doubt that the source of coatli/*L. nephriticum* is the Mexican tree *Eysenhardtia polystachya* (Ort.) Sarg.[2] However, this wood does not contain a large amount of an easily water-soluble blue-fluorescent dye.[8−10] Quite on the contrary, it is very rich (ca. 1% dry weight) in a group of rare, nonfluorescing *C*- and *O*-β-glycosyl-α-hydroxydihydrochalcones. To solve this paradox, we isolated from *E. polystachya* two previously characterized,[9−11] easily

† IQFR.
‡ IQOG.

(1) Monardes, N., *Dos Libros/El uno trata de todas las cosas que traen de nuestras Indias occidentales que sirven al uso de Medicina. . .* , en casa de Sebastian Trugillo, Seville, 1565.
(2) Acuña, A. U.; Amat-Guerri, F. Early History of Solution Fluorescence: The Lignum nephriticum of Nicolás Monardes. *Springer Ser. Fluoresc.* **2008**, *4*, 3–20.
(3) Partington, J. R. *Ann. Sci.* **1955**, *11*, 1–26.
(4) Stapf, O. *Bull. Miscell. Inform. Kew Gardens* **1909**, 293–302.
(5) Möller, H. J. *Ber. Deut. Pharm. Ges.* **1913**, *23*, 88–154.
(6) Safford, W. E. *Ann. Rep. Smithsonian Inst.* **1915**, 271–298.
(7) Sahagún, B. *Matritensis Codex*; Spanish Royal Academy of History, ca. 1560−1564, f203v.
(8) (a) Domínguez, X. A.; Franco, R.; Díaz Viveros, Y. *Rev. Latinoamer. Quím.* **1978**, *9*, 209–211. (b) Burns, D. T.; Dalgarno, B. G.; Gargan, P. E.; Grimshaw, J. *Phytochemistry* **1984**, *23*, 167–169. (c) Álvarez, L.; Rios, M. Y.; Esquivel, C.; Chávez, M. I.; Delgado, G.; Aguilar, M. I.; Villarreal, M. L.; Navarro, V. *J. Nat. Prod.* **1998**, *61*, 767–770.
(9) Beltrami, E.; De Bernardi, M.; Fronza, G.; Mellerio, G.; Vidari, G.; Vita-Finzi, P. *Phytochemistry* **1982**, *21*, 2931–2933.
(10) Álvarez, L.; Delgado, G. *Phytochemistry* **1999**, *50*, 681–687.

10.1021/ol901022g CCC: $40.75
Published on Web 06/17/2009

water-soluble glucosyldihydrochalcones: coatline B (**1**) and coatline A (**3**) (Figure 1). Unexpectedly, it was found that



1 (coatline B): R^1 = *C*-Glu; R^2 = OH
3 (coatline A): R^1 = *C*-Glu; R^2 = H
5: R^1 = H; R^2 = OH; racemate
C-Glu = *C*-β-D-glucopyranosyl

2 (matlaline): R^1 = *C*-Glu; R^3 = H
4: (α*R*,1*S*,3*S*) isomer of **2**
6: R^1 = R^3 = H; racemate
7: R^1 = H; R^3 = Me; racemate

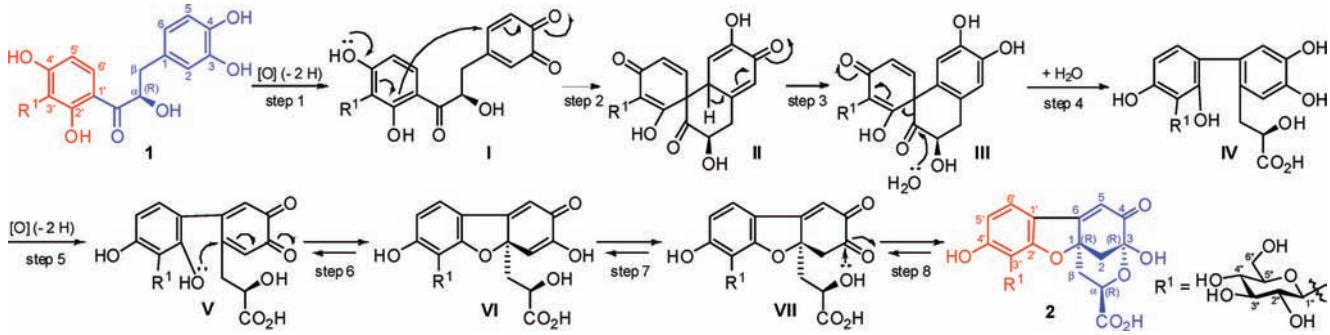
Figure 1. Matlaline (**2**), the fluorophore of *L. nephriticum*, and related compounds.

at room temperature and in slightly alkaline (pH ~7.5) water solution, **1** undergoes a fast, irreversible reaction giving rise to a strongly blue-emitting compound with ca. 100% yield, matlaline (**2**), the fluorophore of the long-sought *L. nephriticum*. No other products were detected in the reaction, even in trace quantities.

The formation of **2**, with a new oxacine core, must result from a sequence of highly directed repetitive reactions (Scheme 1), triggered by the ionization of **1** at the 4′ position in neutral or slightly alkaline medium ($pK'_a = 6.5 \pm 0.2$). The reactive anion can be easily detected as a transient species absorbing at 338 nm if its conversion rate is retarded, e.g., by deoxygenating the solution or lowering the pH. In fact, conversion of **1** into **2** can be prevented in aerated solutions at pH 4 or at pH 8 in the absence of oxygen. The first step of the sequential reaction should be the oxidation of the *o*-diphenol group of **1** by atmospheric dioxygen to yield the highly reactive *o*-quinone **I**. Although in cathechols this process occurs in the presence of oxidants or is catalyzed by enzymes or transition metals,[12,13] we observed full reaction of **1** even in NH_3(gas)-alkalinized ultrapure water solution. In step 2, the C1′-spiro intermediate **II** is formed from the Michael-type intramolecular addition of C-1′ on C-6.[13] Subsequent aromatization to the corresponding 3,4-diphenol **III** (step 3), as that proposed in the enzymatic oxidation of the dihydrochalcone phloridzin,[14] followed by addition of a water molecule to the spirocyclic moiety (step 4), yields compound **IV**, with an α-hydroxypropionic acid substituent. A similar biphenyl derivative was isolated in the phloridzin oxidation noted above,[14] whereas dimerization of a catechol via the corresponding *o*-quinone has been previously observed.[15] The repetition of the oxidative step, now at the 3,4-diphenol group of **IV** (step 5), yields **V**, as well as of the Michael-type intramolecular addition, herein of the 2′-OH group to the *o*-quinone system (step 6), leads to **VI** through a process already observed in some biphenol compounds.[15,16] The tautomerization of **VI** (step 7) produces the keto form **VII**, that yields the final product **2** by α-OH *cis* attack on C-3 (step 8). Under the same conditions, coatline A (**3**) is unreactive, which is consistent with the above reaction sequence. A somewhat similar reaction has been postulated to explain the formation of a related azocine with very low yield.[17]

Matlaline has a molecular formula of $C_{21}H_{22}O_{12}$, 14 atomic mass units more than **1** ($C_{21}H_{24}O_{11}$), and its structure was established from NMR spectroscopic studies. The ^{1}H and ^{13}C NMR spectra of **2** show signals for a *C*-β-D-glucopyranosyl group and two *ortho* aromatic protons almost identical to those of **1**. HSQC and HMBC experiments allow the unambiguous assignment of all the protons and carbons of the tetrasubstituted benzene and its *C*-sugar substituent (see the Supporting Information). The remaining ^{1}H and ^{13}C NMR signals of **2** are assigned to a trisubstituted olefin [δ_H 6.35 (s, 1H, H-5); δ_C 168.9 (C, C-6), 106.7 (CH, C-5)] conjugated with a ketone [δ_C 191.5 (C, C-4)], a hemiacetal carbon [δ_C 94.9 (C, C-3)], a fully substituted sp^3 carbon bearing an oxygen [δ_C 89.0 (C, C-1)], a methylene group without vicinal protons [δ_H 2.54 and 2.47 (1H each, ^2J = 11.2 Hz, 2H-2); δ_C 44.5 (CH_2, C-2)], a (C)CH_2CH(C)O− fragment [δ_H 4.22 (dd, 1H, ^3J = 12.1, 2.8 Hz, H-α), 2.12 and 1.98 (1H each, ^2J = 12.6 Hz, 2H-β); δ_C 70.0 (CH, C-α), 35.9 (CH_2, C-β)], and a carboxyl group [δ_C 177.4 (C)]. Moreover, the C-2 methylene proton at δ_H 2.47 shows a W-type coupling (^4J = 2.8 Hz) with one of the C-β methylene protons (δ_H 1.98), whereas the observed vicinal couplings between the H-α and 2H-β protons (^3J = 12.1 and 2.8 Hz) indicate that H-α possesses an axial orientation. In addition, the HMBC spectrum of **2** displays correlations of H-5 with C-1′, C-1, and C-3, between 2H-2 and C-1, C-3, C-4, C-6, and C-β,

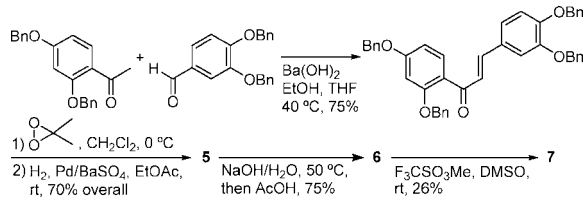
Scheme 1. Plausible Sequential Reaction That Transforms Coatline B (**1**) into Matlaline (**2**) at Room Temperature in Water Solution



and between 2H-β and the carboxyl at δ_C 177.4, as well as with C-1, C-2, C-6, and C-α. The significant HMBC correlation between H-5 and C-1′ [δ_C 109.8 (C)] and all of the above data are only compatible with a structure such as **2** for matlaline. This conclusion was also supported by the synthesis and structural analysis of the aglucon **6** shown below.

The total stereoselectivity shown in Scheme 1 for the conversion of **1** into **2** may deserve further comment. First, since none of the bonds to αC are broken along the reaction sequence, the (α*R*) absolute configuration of **1** is preserved in **2**. However, diastereoisomers (α*R*,1*R*,3*R*)-**2** and (α*R*,1*S*,3*S*)-**4** (Figure 1), instead of the sole isomer **2**, may be expected as reaction products. The observed selectivity could be due to the presence of a *C*-β-D-glucopyranosyl substituent at C3′ and/or to the (α*R*) chiral center in the starting compound **1**. This point was settled by the synthesis of the (α*R*)/(α*S*) racemate **5**, the coatline B aglucon which is also present in *E. polystachya* in enantiopure (α*R*) form.[10] The synthesis of **5** (Scheme 2) was carried out in three steps:

Scheme 2



(1) condensation between 2′,4′-dihydroxyacetophenone and 3′,4′-dihydroxybenzaldehyde, both with their OH-groups appropriately protected as benzyl ethers; (2) epoxide formation in the generated double bond; and (3) regioselective cleavage to the corresponding α-alcohol by hydrogenation, with simultaneous OH-deprotection. The matlaline aglucon **6** and its methyl ester **7** were also prepared from **5**. Single-crystal diffraction analysis of **7** indicated that the crystals contain the racemic mixture of the (α*R*,1*R*,3*R*) and (α*S*,1*S*,3*S*) forms, both presenting the CO_2Me group in an *exo*-equatorial orientation (Figure 2). Thus, the αC chiral center drives the stereoselective transformation of **5** (a racemic mixture) into **6**.

Since the same effect would operate in the transformation of **1** into **2**, total stereoselectivity can be expected for this

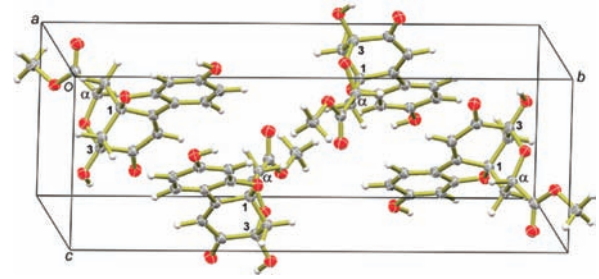


Figure 2. X-ray structure of a racemate **7** single crystal with four molecules in the monoclinic cell unit. From left to right: enantiomers (α*R*,1*R*,3*R*), (α*S*,1*S*,3*S*), (α*R*,1*R*,3*R*), and (α*S*,1*S*,3*S*).

reaction, based on the relative stability of diastereomers **2** and **4**, with the CO_2H substituent in an *exo*-equatorial (**2**) or an *endo*-axial (**4**) orientation relative to the 2-oxabicyclo-[3.3.1]non-6-ene system. From a steric point of view, **2** is expected to be more stable than **4**. In addition, a stabilizing intramolecular H-bond can be formed in isomer **2** between the *cis*-oriented CO_2H and 3-OH groups. Should isomer **4** be formed via the (1*S*) isomer of **VI**, generated by attack of the ionized 2′-OH of **V** from the *Si*-face of its *o*-quinone ring, it could give rise to the more stable isomer **2** through hemiacetal ring-opening, tautomerization and retro-Michael reactions (back-steps 8, 7 and 6, respectively), subsequent attack of the ionized 2′-OH from the *Re*-face of the *o*-quinone ring, and final hemiacetal closure (Scheme 1).

The most conspicuous property of **2** is, of course, the intense fluorescence (λ_f = 465 nm) of even much diluted aqueous solutions. This is due to the combination of a large absorption coefficient in the visible range (Table 1) and a

Table 1. Absorption Properties and pK'_a Values of Coatline B (**1**) and Matlaline (**2**) in Water Solution

compd	pK'_a	solution pH	λ_{ab} (nm) (ε) (M^{-1} cm^{-1})
1	6.5 ± 0.2	4−7	282 (23630 ± 300)
			325 (15240 ± 200)
		10[a]	338 (21700 ± 600)
2	5.5 ± 0.4	4–5.5	307 (5800 ± 600)
			382 (14800 ± 200)
		9	283 (4400 ± 300)
			429.5 (33800 ± 800)

[a] Deoxygenated.

ca. 100% fluorescence quantum yield (Figure 3). The fluorescence intensity of the aqueous solution depends strongly on pH, as noticed also in historical times by Kircher and Boyle,[2] due to the protolytic equilibrium between an emitting and a dark form (p$K'_a \approx 5.5$). In neutral or slightly alkaline solution, the dominant species is the strongly fluorescent dianion form of **2**, in which both the 4′-OH and the CO_2H groups are ionized. Interestingly, two resonant structures can be drawn for this species which remind those

(11) At least two *Eysenhardtia* species produce strongly fluorescent infusions: *E. polystachya* and *E. officinalis*. The work described here was carried out with the first species because several of its components have been previously characterized.

(12) (a) Young, D. A.; Young, E.; Roux, D. G.; Brandt, E. V.; Ferreira, D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2345–2351. (b) Dorrestein, P.; Begley, T. P. *Bioorg. Chem.* **2005**, *33*, 136–148.

(13) Guyot, S.; Cheynier, V.; Souquet, J.-M.; Moutounet, M. *J. Agric. Food Chem.* **1995**, *43*, 2458–2462.

(14) Le Guernevé, C.; Sanoner, P.; Drilleaub, J.-F.; Guyot, S. *Tetrahedron Lett.* **2004**, *45*, 6673–6677.

(15) (a) Guyot, S.; Vercauteren, J.; Cheynier, V. *Phytochemistry* **1996**, *42*, 1279–1288. (b) Tanaka, T.; Mine, C.; Inoue, K.; Matsuda, M.; Kouno, I. *J. Agric. Food Chem.* **2002**, *50*, 2142–2148.

(16) Matsuo, Y.; Tanaka, T.; Kouno, I. *Tetrahedron* **2006**, *62*, 4774–4783.

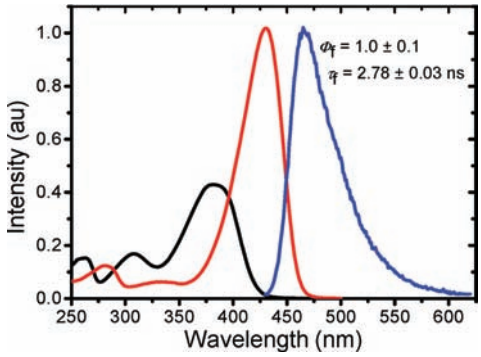


Figure 3. Spectral properties of matlaline (**2**) in water solution. Absorption (red) and corrected fluorescence (blue) spectra, emission yield (Φ_f), and lifetime (τ_f) of the dianion form at pH 9; absorption spectrum of the nonemitting form (black) at pH 4.

of the strongly emitting fluorescein dianion. In contrast, the monoanion form (CO_2^-) of **2** is nonfluorescent and shows UV-shifted weaker absorption (Figure 3). In addition to that, the chemical conversion of **1** into **2** is abolished in water at pH close to 5. All these pH-dependent effects may have confounded the botanical search.[18]

By preparing **6**, the aglucon form of **2**, we also could determine that the emitting properties of **6** are identical to those of matlaline, within experimental error, and independent of the 3′-glucosyl residue, which of course accounts for the large water solubility of **2**. Thus, the fluorescence of the plant infusion originates not only from **2** but also from compounds with the same chromophore but containing a different glycoside group, which can also be formed from the other two *C*- and *O*-*β*-xylopyranosyl α-hydroxydihydrochalcones present in *E. polystachya*.[9,10]

(17) Crescenzi, O.; Napolitano, A.; Prota, G.; Peter, M. G. *Tetrahedron* **1991**, *47*, 6243–6250.

(18) In fact, Monardes already noticed that 0.5 h was needed for the *L. nephriticum* infusion to develop its full blue "color", indicating that he was handling *Eysenhardtia* wood.

It was known very early[6,19] that the infusion of the Philippine *Pterocarpus indicus* (narra tree), used traditionally for kidney diseases, shows a striking fluorescence similar to that of *L. nephriticum*. Moreover, it has been suggested[5,6,20] that a *Pterocarpus* species was the source of, or at least a medicinal substitute for, the Mexican wood. We investigated samples of several *Pterocarpus* species collected in Mexico (see the Supporting Information), but none of them contained a substantial amount of any water-soluble blue-emitting compound. We also analyzed samples of *Pterocarpus indicus* Willd. from the Philippines to find out that its wood contains large quantities of the strongly emitting compound **2** already preformed, but only trace amounts of **1**.[21] Interestingly, the presence of **2** in the wood of *P. indicus* has not been reported in previous studies of the tree's components.[22]

Acknowledgment. We thank S. Castroviejo (Real Jardín Botánico, CSIC, Madrid) for botanical advice, V. Hornillos (Instituto de Química Orgánica General, CSIC) for helpful discussions, and the Ministerio de Educación y Ciencia (Spain), and CSIC for financial support and a I3P fellowship (P.M.).

Supporting Information Available: General experimental procedures, isolation of compounds **1**−**3**, preparation of **5**−**7**, characterization data, and X-ray crystallographic data of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901022G

(19) Delgado, J. J. In *Historia de Filipinas*, written in 1754. Published for the first time in *Historia General Sacro-profana y Natural de las Islas del Poniente, llamadas Filipinas*; Martínez de Zúñiga, Ed.; Manila, 1892; pp 415−416.

(20) Muyskens, M. J. *J. Chem. Educ.* **2006**, *83*, 765–768.

(21) Since the fluorescence of the infusion of both *E. polystachya* and the Philippine *P. indicus* is identical, it is not unlikely that both trees were interchanged as a commercial source of *L. nephriticum*, but at a much later date than that of Monardes' observation, when regular trade with the Philippine Islands was eventually established.

(22) (a) Cooke, R. G.; Rae, I. D. *Aust. J. Chem.* **1964**, *17*, 379–384. (b) Gonzales, E. V. *Philippine J. Sci.* **1978**, *105*, 223–233. (c) Ragasa, C. Y.; De Luna, R. D.; Hofilena, J. G. *Nat. Prod. Res.* **2005**, *19*, 305–309.