Fluorescence of the diterpenoid alkaloids lappaconitine and *N*-deacetyllappaconitine in acetonitrile

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The absorption, excitation, and fluorescence spectra of the diterpenoid alkaloids, lappaconitine and *N*-deacetyllappaconitine, in acetonitrile were studied. On the basis of the coincidence of the spectra with the analogous spectra of model compounds, methyl esters of anthranilic and *N*-acetylanthranilic acids, the conclusion was drawn that the —OOC(Ph)NRH groups are fluorochromes in the alkaloids studied. The high quantum yield of fluorescence and the bathofluoric shift in the luminescence and absorption spectra of *N*-deacetyllappaconitine were explained by an increase in the electron-releasing ability of the —NRH group in the deacetylation of lappaconitine.

Key words: diterpenoid alkaloids, lappaconitine, *N*-deacetyllappaconitine, fluorescence, UV spectra, fluorescence spectra.

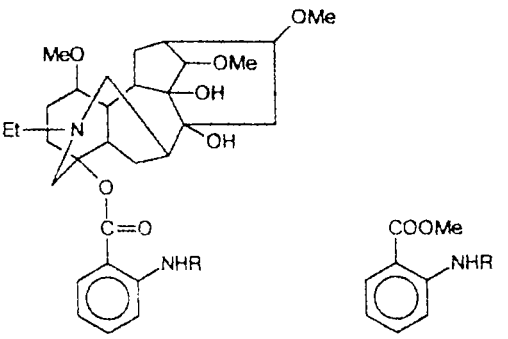
Fluorescence (FL) and chemiluminescence are widely used in biology and medicine.[1,2] The luminescence of aqueous solutions of *lignum nephrilicum* wood extract can be considered as one of the first observations of FL.[3] However, FL of vegetable extracts can be due to small impurities rather than to the main substance.[3]

In a mixture of alkaloids, the correct separation of their contributions to FL would be favored by identification of their fluorochromes produced by synthetic procedures.

The diterpenoid alkaloid *N*-deacetyllappaconitine (**1**), which is present as an impurity in the drug allapinine and whose active component is the alkaloid lappaconitine (**2**), has been detected and identified by fluorescence spectroscopy.[4] This allowed development a procedure for the quantitative determination of **1** in allapinine. However, the identification of the fluorophores responsible for the FL of these compounds has remained open.

The aim of the present work was to study the FL and identify the fluorophoric groups in **1** and **2**. Since numerous organic compounds are known to exhibit intense FL when electron-releasing substituents are introduced into the π-system bonded to the carbonyl group,[5] one could assume that the —OOC(Ph)NHR group is a fluorophore in the alkaloids studied.

The coincidence of the absorption, excitation, and fluorescence spectra of the model compounds, methyl esters of anthranilic acid (**3**) and *N*-acetylanthranilic acid (**4**), with the spectra of **1** and **2**, respectively, would confirm this assumption.



1, 2 **3, 4**

R = H (**1**, **3**), Ac (**2**, **4**)

Experimental

Lappaconitine was isolated by alcoholic extraction of the roots of *Aconitum septentrionale Koelle* and purified by repeated recrystallization from ethyl acetate and acetone.[6] *N*-Deacetyllappaconitine was obtained by hydrolysis of lappaconitine in a 10% solution of H_2SO_4 and purified by recrystallization from acetone.[7] The purity of **1** and **2** was monitored by TLC on Silufol UV_{254} (Kavalier) (ether—pentane—ethanol—ammonia mixture, 20 : 8 : 2 : 0.1). The physico-chemical characteristics of **1** and **2** were identical to those described in the literature.[6] Methyl esters of anthranilic and *N*-acetylanthranilic acids were obtained by methylation of the samples of the corresponding acids with diazomethane in ether of chemically pure grade. The UV spectra were recorded on a Specord M-40 spectrophotometer, the fluorescence spectra were recorded on a Hitachi MPF-4 spectrofluorimeter.

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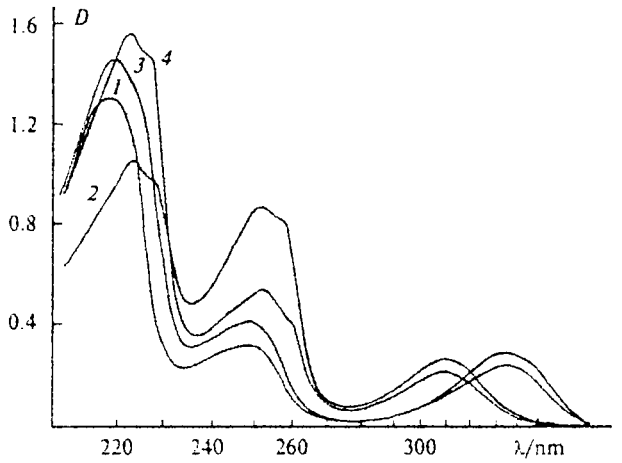

Fig. 1. Absorption spectra of **1** (*1*), **2** (*2*), **3** (*3*), and **4** (*4*). $C = 2 \cdot 10^{-5}$ mol L^{-1}, MeCN, $l = 1$ cm, $T = 295$ K.

Results and Discussion

The absorption spectrum of **2** in MeCN is characterized by three bands (λ_{max}/nm: 223.1, 252.7, and 310.4) and coincides with the spectrum of **4** (Fig. 1). A hypsochromic shift of two short-waves bands (λ_{max}/nm: 219.0 and 249.1) and a bathochromic shift of the long-wave absorption band (λ_{max}/nm: 338.1) with respect to lappacotinine (see Fig. 1) is observed in the UV spectrum of **1**; although it is identical to the spectrum of **3**. The coincidence of the UV spectra of alkaloids **1** and **2** with the spectra of esters **3** and **4**, respectively, points to the fact that the absorption in the compounds investigated is due to the —OOC(Ph)NHR chromophore.

The introduction of electron-releasing substituents to the phenyl radical is known to be accompanied by an appreciable long-wave shift in the absorption spectra caused by their interaction with the carbonyl group.[8,9] Therefore, deacetylation of **2** and **4** results in an increase in the electron-donor properties of the amino group and, as a consequence, in a bathochromic shift of the long-wave absorption band in the spectra **1** and **3**. In this connection, one can assume that the absorption band at the longest wavelength is associated with intramolecular charge transfer caused by the interaction between the —NHR and the carbonyl groups.

Although the absorption spectra of **1** and **2** in MeCN are nearly identical (except for the bathochromic shift of the long-wave band by 28 nm), which hampers the quantitative determination of **1** in allapinine, the distinctions in the excitation spectra are more appreciable (Fig. 2). Three maxima (at λ_{max}/nm: 260, 315, and 360) are observed in the FL excitation spectrum of lappaconitine, whereas two maxima are observed for *N*-deacetyllappaconitine (at λ_{max}/nm: 260 and 350). In Fig. 2, the uncorrected FL excitation spectra of compounds **1**—**4** are shown, which is likely the reason for their mismatch with the absorption spectra.

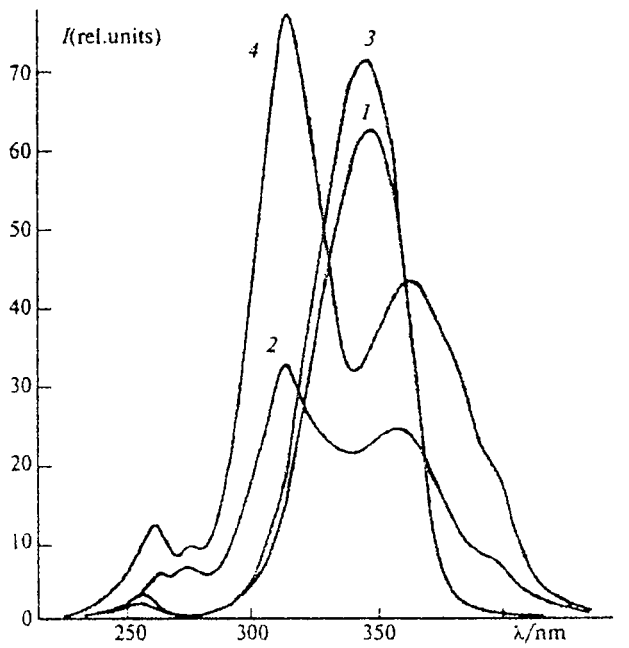

Fig. 2. Excitation spectra of **1** (*1*), **2** (*2*), **3** (*3*), and **4** (*4*). $C = 2 \cdot 10^{-5}$ mol L^{-1}, MeCN, $T = 295$ K, $\Delta\lambda = 1$ nm.

Nevertheless, the FL excitation spectra of **1** and **2** (analogously to the UV spectra) are identical to the spectra of the corresponding fluorophores **3** and **4** (see Fig. 2).

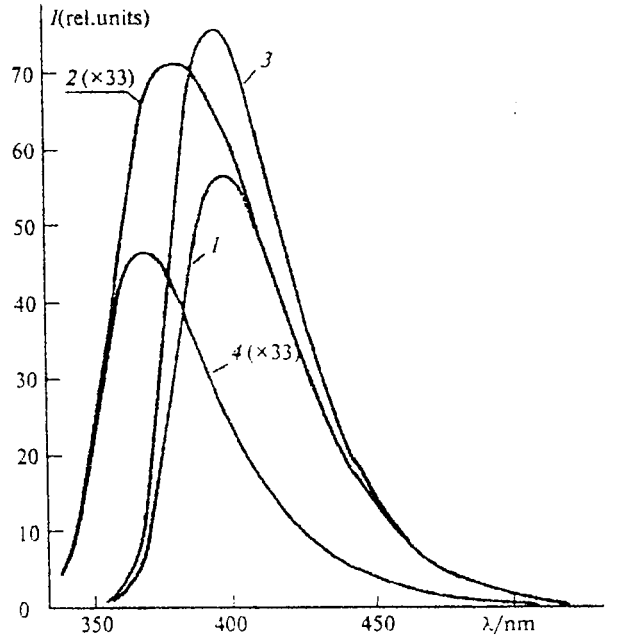

Fig. 3. Fluorescence spectra of **1** (*1*), **2** (*2*) ($\lambda_{excit} = 350$ nm), **3** (*3*), and **4** (*4*) ($\lambda_{excit} = 315$ nm). $C = 2 \cdot 10^{-5}$ mol L^{-1}, MeCN, $T = 295$ K, $\Delta\lambda = 1$ nm.

Both alkaloids exhibit intense fluorescence. The FL maxima of **1** and **2** are observed at 400 and 382 nm, respectively (Fig. 3). In addition to a bathochromic shift, the intensity of chemiluminescence of **1** is higher than that of FL of **2** by a factor of ~25, which defines the high sensitivity of the spectral-luminescent method when the content of *N*-deacetyllappaconitine in allapinine is determined. However, if the position of the FL maximum of **1** coincides with that of **3**, a bathofluoric shift is observed in the spectrum of **2** with respect to **4** (see Fig. 3). The distinctions in the fluorescence spectra of **2** (λ_{max}/nm: 382) and **4** (λ_{max}/nm: 370) are likely due to a small impurity of **1**. Thus, a comparison of the spectral-luminescent characteristics of **1** and **2** shows that deacetylation of **2**, which results in an increase in the electron-releasing effect of the carbonyl group, is accompanied by both an increase in the intensity of the glow and a bathofluoric shift of the FL maximum of **1** similar to the long-wave bands in the absorption spectra. Analogous changes were previously detected in studies of the luminescence spectra of naphthalimide derivatives[5] and explained by the increased polarity of the carbonyl group owing to the introduction of amino or acetylamino groups.

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