ORGANIC
LETTERS

2000
Vol. 2, No. 15
2311−2313

Berberine Cation: A Fluorescent Chemosensor for Alkanes and Other Low-Polarity Compounds. An Explanation of This Phenomenon

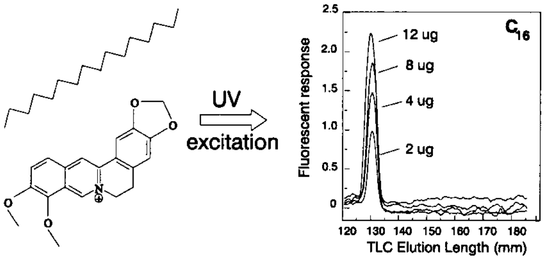
Fernando P. Cossío,*,[†,§] Ana Arrieta,[†] Vicente L. Cebolla,*,[‡] Luis Membrado,[‡] Jesús Vela,[||] Rosa Garriga,[‡] and María P. Domingo[‡]

Kimika Fakultatea, Euskal Herriko Unibertsitatea, P.K. 1072, 20080 San Sebastián-Donostia, Spain, Instituto de Carboquímica, CSIC, María de Luna, 12, 500015 Zaragoza, Spain, and Grupo de Espectroscopía Analítica y Sensores, Departamento de Química Analítica, Facultad de Veterinaria, Universidad de Zaragoza, 50013 Zaragoza, Spain

vcebolla@carbon.icb.csic.es

Received May 18, 2000

ABSTRACT



Alkanes in the presence of berberine sulfate provide an enhancement of fluorescent signal, which depends on alkane concentration and structure, when the system is irradiated with monochromatic UV light. Computational analysis suggests that an ion-induced dipole between alkanes and berberine sulfate is responsible for this phenomenon. This interaction can properly model the experimentally obtained fluorescent response. The proposed explanation allows other interacting systems to be designed, which have been experimentally confirmed.

Chemosensors are small molecules that are selectively able to bind the analyte of interest with a measurable change in a property of the system (i.e., fluorescence spectra).[1] Development of sensors for alkanes would be of interest for petrochemistry, and environmental and geological sciences, although the fact that alkanes have classically been considered almost inert molecules has precluded the research in this field. Likewise, it has been reported for a long time that the fluorescence of a broad variety of solutes adsorbed on thin-layer chromatography plates can sometimes be greatly enhanced by revealing the plates with paraffinic compounds, without any apparent chemical reaction, to improve sensitivity and reduce their detection limits.[2] Nevertheless, attempts to explain these data in order to predict new analytical systems of interest were scarce and did not deal with the phenomenon at a molecular level. When berberine sulfate (impregnated onto a silica gel plate) is in the presence of an alkane, and the system is irradiated with long-wavelength UV light, we found that a fluorescent signal is produced

---

[†] Euskal Herriko Unibertsitatea.

[‡] CSIC.

[§] E-mail: qopcomof@sc.ehu.es.

[||] Universidad de Zaragoza.

(1) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.

(2) Baeyens, W.R.G. In *Fluorescence and Phosphorescence of Pharmaceuticals, in Molecular Luminiscence Spectroscopy. Methods and Applications: Part 1*; Schulman, S. G., Ed.; Vol 77 in Chemical Analysis (Elving, P. J., Winefordner, J. D., Eds.), Wiley Interscience: New York, 1985; Chapter 2.

10.1021/ol006075p CCC: $19.00

which depends on the alkane concentration and on the alkane length[3,4] (Figure 1). The addition of each new -CH_2- group results in an increase in the fluorescent response. For analytical purposes, the sensitivity of the determinations can be tailored through the choice of adequate impregnation conditions (impregnation time of the plates and concentration of berberine).

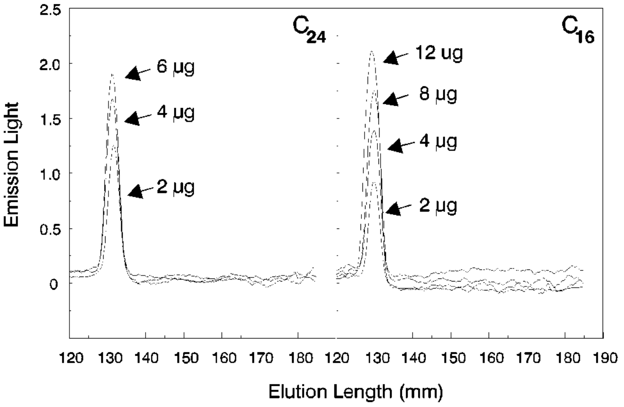


Figure 1. Chromatograms of different sample loads of *n*-tetradecane (C_{24}) and *n*-hexadecane (C_{16}) determined by enhancement of fluorescent response using berberine-impregnated TLC silicagel plates.

An explanation, based upon experimental findings and computational studies, is presented here to explain the reasons underlying the fluorescence signal. In a previous work,[3] our calculations suggested that there is an electrostatic interaction between the alkane molecule and the electron-deficient π-system of berberine and that there are van der Waals contacts in several positions. We have now found[5] that an ion-induced dipole interaction between alkanes and berberine sulfate can properly model this enhancement of fluorescence. We based our conclusion on the following data. We first performed a complete geometry optimization of the berberine cation using the PM3 semiempirical Hamiltonian at the Hartree−Fock (HF) level. From these data, it can be concluded that berberine is a near planar cation with a remarkable conformational rigidity. Therefore, explanations of the berberine−alkane interactions based upon conformational changes were discarded. We also optimized at the HF/PM3 level the ion−molecule complex between *n*-hexane and the berberine cation. *n*-Hexane was used for simplification of calculations. Our results also indicate that neither the HOMO−LUMO gap nor the composition of the frontier molecular orbitals is affected by the interaction with the alkane. We have also optimized the geometries of several alkane−berberine complexes using the AMBER force field. Monte Carlo simulations were performed in order to locate the optimal conformation for each system. Our results indicate a considerable conformational rigidity, thus suggesting that the response for different alkanes must not be due to significant changes in the berberine geometry on passing from one alkane to another. We next optimized the geometries of berberine and the berberine−*n*-hexane complex in the first excited state at the 3X3CI-HE/PM3 level of theory. The calculations show that an $S_0 \rightarrow S_1$ transition induces considerable variation in the berberine geometry in both cases, mainly because of the antibonding C−N interaction in berberine structure. According to these results, the enhancement of intensity observed in the berberine−alkane systems can be explained by considering two factors. On one hand, the intensity of the emitted signal is proportional to the Einstein coefficient of espontaneous emission A,[6] which depends inversely on the dielectric constant of the medium. A lipophilic compound (e.g., an alkane) that interacts with berberine and surrounds it creates an apolar microenvironment which, in turn, lowers the dielectric permittivity of the silica gel−berberine medium, thus enhancing the intensity of the fluorescence signal. On the other hand, the enhancement of intensity of a given fluorescent signal is directly related to the quantum yield ϕ.[7] According to previous studies reported by us,[3] another function of the apolar neutral molecule is to protect the fluorophore from polar nonradiative decays. Therefore, ϕ must be proportional to the solvation energy[8] between the fluorescent cation k and the apolar neutral molecule i:

$$\Phi_{\mathrm{ik}} = \mathrm{U}_k \frac{e^2 Z_{\mathrm{k}}^{\,2}}{8\pi\epsilon r^4}\alpha_{\mathrm{i}}$$

where U_k is the proportionality constant at a given temperature, Z_k is the charge of the fluorescent cation (in the case of berberine $Z_k = 1$), r is the distance from the charge to the center of the induced dipole, ϵ is the dielectric permittivity of the mediun, and α_i is the intrisic polarizability of the organic compound. According to this expression, for a given molecule i the value of ϕ_{ik} depends only upon the value of α_i at a given temperature, since the remaining magnitudes must be practically constant on passing from one alkane to another. Therefore, under these conditions and at a given concentration, the enhancement of intensity is linearly

---

(3) (a) Cossío, F. P.; Arrieta, A.; Cebolla, V. L.; Membrado, L.; Domingo, M. P.; Henrion, P.; Vela, J. *Anal. Chem.* **2000**, *72*, 1759−1766. (b) Cebolla, V. L.; Membrado, L.; Domingo, M. P.; Henrion, P.; Garriga, R.; González, P.; Cossío, F. P.; Arrieta, A.; Vela, J. *J. Chromatogr. Sci.* **1999**, *37*, 219

(4) Although this phenomenon also takes place in solution, the use of silica gel plates provides a rigid support that enhances the fluorescent response and also a chromatographic system to separate the analyte of interest from other components in complex mixtures. Moreover, the signal can be easily quantified using a fluorescence scanning densitometer (in this case, a CS9301 TLC scanner from Shimadzu, Japan).

(5) Procedure details can be found in ref 3a. PM3 semiempirical Hamiltonian at the Hartree−Fock (HF) level was used for a complete geometry optimization of the berberine cation. The ion−molecule complex between *n*-hexane and the berberine cation was optimized at the HF/PM3 level. Finally, geometries of berberine and the berberine−*n*-hexane complex in the first excited state were optimized at the 3X3CI-HE/PM3 level of theory.

(6) Atkins, P. W.; Friedman, R. S. *Molecular Quantum Mechanics*; Oxford University Press: Oxford, 1997; p 508.

(7) Lakowitz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983; pp 9−11.

(8) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*; VCH: Wheinheim, 1990; pp 9−13.

dependent on the polarizability of the neutral molecule surrounding the fluorophore:

$$\Delta I_{\mathrm{fi}} = A_{\mathrm{f}} + B_{\mathrm{f}}\alpha_{\mathrm{i}}$$

where A_f and B_f are constants for a given fluorophore and α_i is the polarizability of the organic molecule. This model allows the influence of alkane chain length on fluorescence intensity to be explained. Thus, our data show that there exists a linear regression between the fluorescent response and the polarizability of *n*-alkanes (Figure 2).[9]

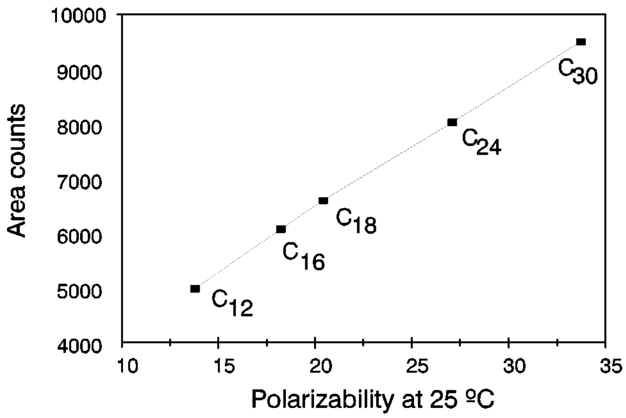


Figure 2. Chromatographic response in area counts versus polarizability for several *n*-alkanes (40 μg injected).

Our explanation suggest that other interesting analytical compounds which have poor ultraviolet or fluorescent responses could also interact with berberine cation and provide fluorescence response.[3] This possibility has been experimentally confirmed. Thus, neutral lipids (e.g., stearic acid, oleic acid, 5α-cholestane, 5*β*-cholestan-3α-ol, cholesterol, stearylcholesterol), prostaglandin VII, polar lipids (a ceramide, a galactocerebroside, phospholipids), cholecalciferol (vitamin D3), propilenglycol, 18-crown-6 ether, geraniol, and octylbenzene have been detected up to now using commercial thin-layer chromatographic systems. The study of the relative responses of these compounds should provide interesting complementary data and probably reflect the influence of their polarities.

In conclusion, an organic compound which significantly lowers the dielectric constant of the silica gel-impregnating agent system, and gives an adequate quantum yield, will produce a fluorescence enhancement. Our experimental results confirm the validity of this model. Thus, we have found a linear dependence between the increment of the fluorescent response and the polarizability of the organic compounds studied ($r = 0.999$, Figure 2).[9] The explanation proposed in this work may be general for the cases of fluorescence enhancement in which there is no apparent chemical reaction. Concerning this point, research is in progress in our laboratory.

Acknowledgment. This work was supported by the Plan Nacional de I+D (Spanish CICYT, project QUI98-0852) and the Gobierno Vasco/Eusko Jaurlaritza (project GV 170.215-EX97/11).

OL006075P

---

[9] Chromatographic conditions for Figure 2 were as follows: applied volume, 0.4 μL; impregnation conditions, 4 mg of berberine sulfate in 200 mL of methanol during 20 s; elution, *n*-hexane (9-min). Fluorescent scanning densitometry after excitation at 365 nm was performed (1 × 1 mm beam size), and emission was collected in the 450−550 nm zone. The corresponding polarizabilities for the alkanes were calculated from Clausius-Mossotti equation (Debye, P. *Polar Molecules*; Dover Publications, 1929; p 11). Data were obtained from TRC database (Texas A&M University).