# 7-OXO-7*H*-BENZIMIDAZO[2,1-*a*]BENZ[*d*,*e*]ISOQUINOLINE-9-SULPHONYL CHLORIDE AS A NEW FLUORESCENCE REAGENT FOR IDENTIFICATION AND DETERMINATION OF ALIPHATIC AMINES

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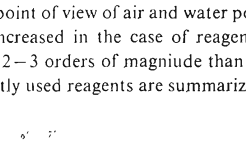
Received March 17th, 1982

Absorption and fluorescnece properties have been studied of a new fluorescence reagent -7-oxo--7*H*-benzimidazo[2,1-*a*]benz[*d*,*e*]isoquinoline-9-sulfphonyl chloride and the corresponding sulphonamides derived from aliphatic amines. The fluorescence intensity is temperature-independent in the range 0–35°C and depends linearly on concentration of the sulphonamides in the range $6 . 10^{-9}$ to $1 . 10^{-6}$ mol $l^{-1}$. TLC separation of the sulphonamides is good, the yields of elution being 95%, which allows application of the procedure to determination of the amines.

Synthesis and application of new reagents for analytical identification and determination of various types of organic compounds of low concentrations is particularly important from the point of view of air and water pollution control. Sensitivity of detection is especially increased in the case of reagents possessing fluorescence properties, being higher by 2–3 orders of magniude than that of the reagents based on colour reactions. Currently used reagents are summarized in refs[1,2].



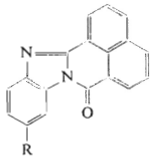

The newly prepared reagent, 7-oxo-7*H*-benzimidazo[2,1-*a*]benz[*d*,*e*]isoquinoline--9-sulphonyl chloride (1,2-naphthoylenebenzimidazole-6-sulphonyl chloride) (*II*), reacts easily with primary and secondary amines to give products possessing intensive fluorescence. Preparation of the reagent and its derivatives was described in ref.[2]. The present communication deals with absorption and fluorescence properties of the reagent and its derivatives with primary aliphatic amines and with possibility of determination of aliphatic amines in mixtures after transformation to fluorescent

derivatives and TLC separation. Determination of amines by means of HPLC will be dealt with in a next communication.

## EXPERIMENTAL

### Spectral Measurements

The absorption spectra were measured with recording spectrophotometers Specord UV VIS and Perkin–Elmer 356 equipped with a device for data collecting on punched tape. The absorbance values were introduced from the spectrophotometers to the punched tape in 2 nm intervals. Calculation of log $\varepsilon$ was carried out with Hewlett–Packard 2116B computer.



*I*, R = H
*II*, R = $SO_2Cl$
*III*, R = $SO_2NHCH_3$
*IV*, R = $SO_2NHC_2H_5$
*V*, R = $SO_2NHCH(CH_3)_2$
*VI*, R = $SO_2NH(CH_2)_3CH_3$
*VII*, R = $SO_2NH(CH_2)_7CH_3$
*VIII*, R = $SO_2NH(CH_2)_9CH_3$
*IX*, R = $SO_2N(C_2H_5)_2$

The absorption spectra of the studied compounds *I*—*IX* were measured in ethanol and benzene. Purity of the reagent and its derivatives was checked by measuring the excitation spectra of fluorescence and comparing them with the absorption spectra; the used concentration was $2 \cdot 10^{-6}$ . mol $l^{-1}$, the range of the excitation spectrum being from 260 nm to 470 nm at the wavelength of fluorescence 370 nm and 480 nm in benzene and ethanol, resp.

The fluorescence spectra, excitation fluorescence spectra, and the quantum yields of fluorescence were measured with a recording spectrophotometer Hitachi Perkin–Elmer MPF-2A modified for data collecting on punched tape. The corrected fluorescence spectra and quantum yields of fluorescence were calculated by means of special programs with the above-mentioned computer. The fluorescence spectra were measured in ethanol and benzene; concentration of the solutions was the same as in the measurements of the excitation spectra. Such low concentration already excludes the reabsorption and inner filter effects. The fluorescence spectra were measured in the region 400—600 nm at the wavelength of excitation radiation equal to 382 nm and with 10 nm width of the excitation and emission slot. The spectra were taken from the middle of a 1 × × 1 cm cell.

The quantum yields of fluorescence were measured by the relative method[3] using the relation

$$\Phi = ER(I/S)/\sigma A\,, \qquad (1)$$

where

$$\sigma = E_{QS}R_{QS}(I/S)_{QS}/\Phi_{QS}A_{QS}\,, \qquad (2)$$

$\Phi$ stands for quantum yield of fluorescence of the compound measured, $A$ is absorbance of the solution measured, $(I/S)$ is integral fluorescence intensity of the substance measured related to the fluorescence intensity of an auxiliary standard (application of the ratio $(I/S)$ eliminates variation of sensitivity of the apparatus), $R$ is a factor correcting the dependence of fluorescence intensity on refractive index of the solution measured[4,5], $E$ is a factor correcting spectral dependence of intensity of the excitation radiation, $\sigma$ is a constant of the apparatus. The index $QS$ denotes quinine sulphate solution in 0·5M-$H_2SO_4$ whose quantum yield of fluorescence 0·546 was taken as the basis of the measurements[6]. As an auxiliary standard for measurement of signal $S$ served $1 \cdot 10^{-5}$ mol $l^{-1}$ solution of compound $I$ in ethylene glycol which exhibits very good photostability in the excitation to the 1. absorption band[4]. The integral intensity of fluorescence was calculated from digital record of the spectrum corrected with respect to spectral dependence of sensitivity of the emission part of the spectrophotometer.

The temperature dependence of fluorescence was measured with a benzenic solution of compound *IV* of $2 \cdot 10^{-6}$ mol $l^{-1}$ concentration in temperature range from 0 to 30°C.

The concentration dependence of fluorescence intensity $I_\lambda$ at the wavelength connected with no reabsorption can be expressed by Eq. (*3*) for the used experimental arrangement.

$$I_\lambda = kI_0 \cdot 10^{-\varepsilon c l_x}(1 - 10^{-\varepsilon c \Delta l_x}) \qquad (3)$$

$\varepsilon$ means reabsorption coefficient, $c$ is concentration, $l_x$ is the solution thickness which is crossed by excitation light before entering the scanned region, $\Delta l_x$ is magnitude of the scanned region in the direction of the excitation beam. The k constant involves, inter alia, the value of absolute quantum yield and geometry conditions of the fluorescence measurement. For sufficiently small values of the product $\varepsilon c l_x$ the relation is simplified to

$$I_\lambda = 2{\cdot}3kI_0\varepsilon c\, \Delta l_x\,, \qquad (4)$$

and the fluorescence intensity will be linear function of concentration. The deviation from linearity at higher $\varepsilon c l_x$ values which is due to the inner filter effect was corrected by the method[5]

Fluorescence Determination of Ethylamine by means of TLC

Preparation and isolation of the crystalline derivatives were described in ref.[2], and the elution data are given in ref.[7].

*In situ*. The method was checked with the use of benzenic solution of compound *IV* with concentrations $c_{IV} = 2 \cdot 10^{-6}$ to $1{\cdot}6 \cdot 10^{-5}$ mol $l^{-1}$. The solution (2 µl) of given concentration was introduced on thinn layer (Silufol, Kavalier ČSSR) and the chromatogram was developed by ascendent technique using benzene–ethyl acetate 2 : 1 and 8 : 3. Diameter of the spots was $0{\cdot}5 \pm 0{\cdot}1$ cm. Fluorescence intensity ($I_F$) of the spot was measured with a fluorescence spectrophotometer equipped with a special holder enabling to locate the spot on the chromatographic plate in the place of maximum intensity of incident excitation radiation. Filters were used in the measurements to eliminate diffused radiation. Fluorescence intensity of the background was measured along with fluorescence intensity of the spot.

*After elution from the layer*. The above-mentioned solutions (10 µl) were introduced on the thin layer in the form of a band, and the chromatogram was developed by ascending technique using benzene–ethyl acetate 8 : 3 as eluent. The band position was found in UV light, and the corresponding stripe of about 1 cm width was cut off and eluated with acetone (which proved to be the best eluent) in a test tube closed with a ground glass stopper. Yield of the elution was 95%. Acetone was evaporated, and the residue was dissolved in a defined amout of ethanol, and fluorescence intensity of the solution was measured.

TABLE I

Wave numbers $\tilde{\nu}_{max}$ and molar absorption coefficients $\varepsilon_{max}$ of the absorption maxima of the compounds studied

| Compound | Benzene | | Ethanol | |
|---|---|---|---|---|
| | $\varepsilon_{max}$ $l\,cm^{-1}\,mol^{-1}$ | $\tilde{\nu}_{max}$ $\mu m^{-1}$ | $\varepsilon_{max}$ $l\,cm^{-1}\,mol^{-1}$ | $\tilde{\nu}_{max}$ $\mu m^{-1}$ |
| *I* | 12 059 | 2·60 | 13 448 | 2·62 |
| *II* | 20·271 | 2·58 | 17 255 | 2·62 |
| *III* | 9 218 | 2·62 | 12 670 | 2·62 |
| *IV* | 13 031 | 2·60 | 14 946 | 2·62 |
| *V* | 14 759 | 2·62 | 17·612 | 2·60 |
| *VI* | 12 906 | 2·58 | 13·909 | 2·60 |
| *VII* | 7 892 | 2·58 | 9 096 | 2·60 |
| *VIII* | 13 060 | 2·58 | 14 333 | 2·60 |
| *IX* | 14 770 | 2·60 | 13 989 | 2·60 |

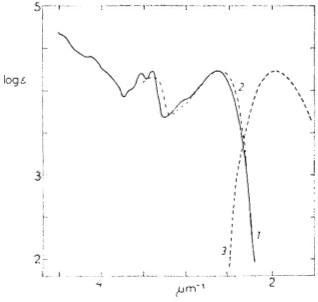



FIG. 1

Absorption, fluorescence, and excitation spectra of ethanolic solution $2 \cdot 10^{-6}$ mol . $l^{-1}$ compound *II* at 298 K. *1* the absorption spectrum, *2* the excitation spectrum at 470 nm wavelength of the excitation radiation, *3* the fluorescence spectrum at 382 nm wavelength of the excitation radiation

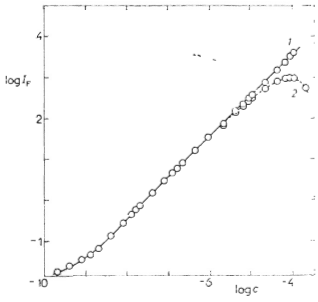



FIG. 2

Concentration dependence of fluorescence intensity of benzenic solution of compound *IV* at 298 K at 382 nm wavelength of the extitation radiation. *1* the corrected course (for the method of correction see the text), *2* the non-corrected course

## RESULTS AND DISCUSSION

The investigation of absorption and fluorescence spectra of the reagent *II* and its derivatives showed that substitution of Cl in *II* by alkylamino group has practically no effect on character of the absorption spectra (Fig. 1 and Table I). Table I gives values of wave numbers and molar absorption coefficients of the first absorption

TABLE II

Wave numbers $\tilde{\nu}_{max}$ of the fluorescence maxima of the compounds studied at the wavelength 382 nm of the excitation radiation

| Compound | $\tilde{\nu}_{max}$, $\mu m^{-1}$ | |
|---|---|---|
| | in benzene | in ethanol |
| *I* | 1·94 | 1·88 |
| *II* | 2·08 | 1·94 |
| *III* | 2·08 | 1·98 |
| *IV* | 2·08 | 1·98 |
| *V* | 2·08 | 1·98 |
| *VI* | 2·08 | 1·94 |
| *VII* | 2·08 | 1·98 |
| *VIII* | 2·08 | 1·94 |
| *IX* | 2·08 | 1·98 |

TABLE III

The fluorescence quantum yields $\Phi$ of the compounds studied

| Compound | $\Phi$ | |
|---|---|---|
| | in benzene | in ethanol |
| *I* | 0·819 | — |
| *II* | 0·730 | 0·540 |
| *III* | 0·828 | 0·650 |
| *IV* | 0·790 | 0·510 |
| *V* | 0·830 | 0·600 |
| *VI* | 0·810 | 0·620 |
| *VII* | 0·770 | 0·580 |
| *VIII* | 0·780 | 0·590 |
| *IX* | 0·780 | 0·620 |

bands, Table II presents wave numbers of maxima of fluorescence band of the investigated compounds in benzene and in ethanol.

From the fluorescence quantum yields given in Table III it can be seen that the values are somewhat higher in benzene than in ethanol. The fluorescence intensity is practically independent of temperature within the range 0–35°C.

The concentration dependence of fluorescence intensity given in Fig. 2 shows linear course within the concentration range of the amide from $6 . 10^{-9}$ to $1 . 10^{-7}$ mol $l^{-1}$. At higher concentrations, the inner filter effect causes deviation from linearity, and at concentrations above $1 . 10^{-4}$ mol $l^{-1}$ even the fluorescence intensity decreases. Using transmissivity values, the experimental curve of dependence of fluorescence intensity on concentration can be corrected[5], and thus the linear region can be extended to higher concentrations. Clearly, the whole given concentration range cannot be measured at the same parameters of the apparatus (*i.e.* voltage in the photomultiplier and slot width).

The fluorescence determination of amines by the *in situ* TLC method revealed that in the apparatus used the fluorescence intensity depended on the spot size and shape, which could affect accuracy of the method. If experimental conditions of the chromatography were adjusted to keep the spot diameter within the limits $0{\cdot}5 \pm 0{\cdot}1$ cm, the *in situ* method with the described experimental arrangement of the fluorescence measurement was practically as accurate as the elution method (for both methods correlation coefficient of linear dependence of fluorescence intensity *vs* concentration was equal to 0·998).

On the basis of the results presented it can be stated that the new fluorescence reagent *II* shows at least comparable properties with those of other fluorescence reagents, its main advantages being: photostability, simple synthesis, high reactivity to primary and secondary amines, and high quantum yield of fluorescence.

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Translated by J. Panchartek.