FLUORESCENCE OF THE TETRACYCLINE-CALCIUM--SODIUM BARBITAL COMPLEX

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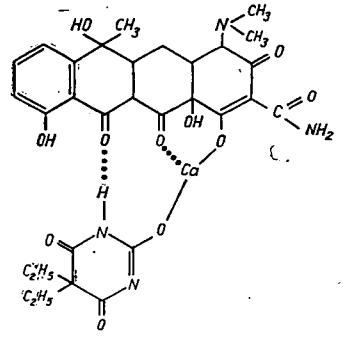
The fluorescence of the complex of tetracycline with calcium ions (Ca^{2+}) and sodium diethylbalbiturate (sodium barbital), extracted into ethyl acetate, is studied in the concentration range from 10^{-5} to $1.3 \cdot 10^{-4}$ mole/l. The intensity of fluorescence is found to decrease in time *i.e.* as the sample ages. However, the spectrum emitted is independent of time and independent of the excitating wavelength and tetracycline concentration.

Introduction

Tetracycline (TC) easily forms complexes with the cations of numerous bi-valent and tri-valent metals. Moreover, it forms mixed complexes with 5,5-disubstituted derivatives of barbituric acid, and calcium ions (Fig. 1, complex II) [1]. Under well-defined conditions of pH 8.9, complex II can be extracted quantitatively with ease when applying certain organic solvents *e.g.* ethyl acetate. On excitation with the Hg line at 365 nm, the extracts exhibit a strong luminescence.

The investigation of its luminescent properties is not only a topic of interest in itself, but it is highly useful from the therapeutical point of view, as well.



II

Fig. 1. The structure of the complex of tetracycline with calcium ions and sodium barbital (II)

Experimental

When measuring emission spectra, we used a setup consisting of two Zeiss SPM—1 monochromators, an RCA 5819 photomultiplier with a sensitivity maximum at 490 nm, a high voltage supply, and a Zeiss galvanometer.

Fluorescence was excited with the 356 and 405 nm lines of an HBO—50 mercury lamp filtered through a monocrhomator, at an angle of 45° to the direction of observation. For the measurements, a quartz cuvette of 1 mm thickness was used. The emission spectra were corrected for the spectral sensitivity of the photomultiplier and the dispersion of the monochromator prism. With regard to the weak overlap of the absorption and emission spectra, no corrections fo reabsorption were introduced.

Results and discussion

In the present measurements, we applied throughout 0.5 mole/l of barbital in order to obtain unequivocal results [2]. Under the above-stated conditions, we measured the stability in time of the complex at various concentrations of TC. Fig. 2 shows the time-variations in fluorescence of the complex, measured at the maximum of emission at two distinct TC concentrations. The intensities decrease markedly with time, at first rather steeply during several hours, and then at a slower rate and linearly. The experimental conditions were chosen so as to have an initial intensity of fluorescence almost equal to a different TC concentration (this was achieved by adjusting the monochromator slits). This enabled us to compare the rate of the decrease in intensity in case of the various concentrations of TC. At a lower TC concentration, the rate of decrease in fluorescence was thus found to be higher. It is to be stated samples were irradiated at $\lambda = 365$ nm at the moment of measurement only.

We carried out measurements to verify whether the emission recorded at various time-intervals originates in the same luminescent objects. Fig. 3 shows emission spectra, recorded immediately subsequent to the preparation of the solutions and 48 hrs later. The spectra coincide completely.

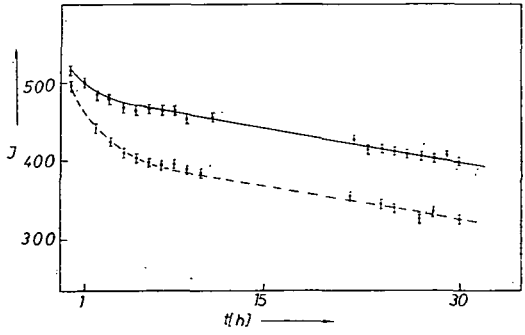


Fig. 2. The fluorescence intensity of tetracycline complex II *vs.* time
Curve ——— for TC concentration $c = 10^{-4}$ M/1;
curve -------- for $c = 2 \cdot 10^{-5}$ M/1

From the preceding results we are drawing the conclusion that, in spite of the slow decomposition of the complex in time, the fluorescence, observed for every moment of time and for each TC concentration, originates in the same species and the decomposition product is non-fluorescent.

Moreover we studied the influence of irradiation on the stability of the luminescent tetracycline complex. The curve of Fig. 4. shows the changes in intensity of fluorescence for a TC concentration of $2 \cdot 10^{-5}$ mole/l, obtained at continuous irradiation with the intense light of a Hg lamp at the exciting wavelength $\lambda = 365$ nm. In this case the decrease in fluorescence is considerable. The intensity of fluorescence decreases to 50 per cent of its initial value after no longer than 1 h of irradiation.

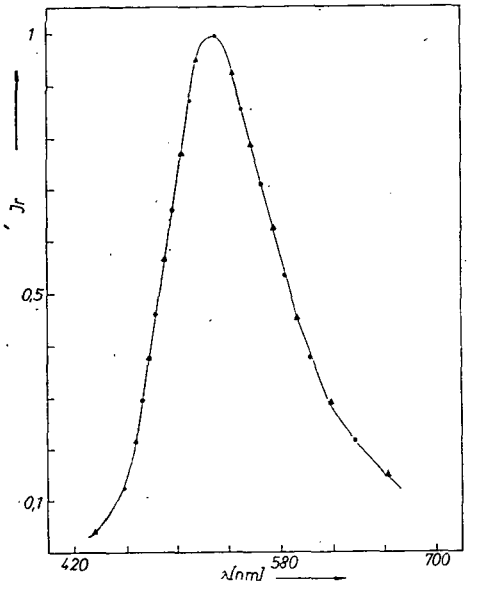


Fig. 3. The emission spectrum of tetracycline complex II recorded at various time intervals (● — immediately and ▲ — after 48 hrs)

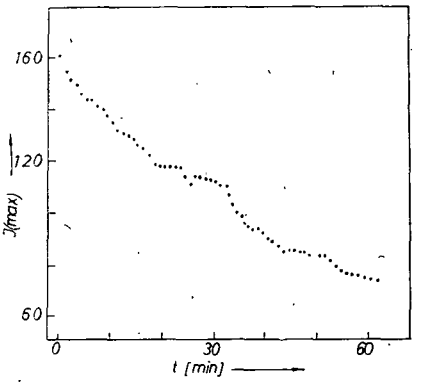


Fig. 4. Decrease in time of the fluorescence intensity of complex II in case of continous irradiation

Additionally, we measured the spectral distributions of complex II, excited with various wavelengths in the region of the long-wave absorption band of the complex. The emission spectra excited with wavelengths 365 nm and 405 nm should be identical with those given in Fig. 3.

Conclusions

From the present results, we draw the following conclusions:

1. The complex of tetracycline, diethyl-barbituric acid, and calcium ions in ethyl acetate is unstable; though its decomposition in time proceeds relatively slowly. Excitation of the complex by irradiating it with light enhances the decomposition rate strongly.
2. The emission spectra are independent of the aging time of the sample, and of the exciting wavelength, proving that, irrespective of the above-stated variables, the fluorescence always originates in the same structurally identical species. Moreover, the decomposition products are non-fluorescent.

References

[1] *Kohn, K. W.:* Anal. Chem. **33**, 862, (1961).
[2] *Regosz, A.:* Dissertation, Medical Academy, Gdansk (1975).

ФЛУОРЕСЦЕНЦИЯ КОМПЛЕКСА ТЕТРАЦИКЛИНА

Е. Гживач, Я. Видуховска и А. Регош

Исследовалась флуоресценция комплекса тетрациклина с барбиталом и кальцием в этилацетате. Установлено, что комплекс тетрациклина является непрочным, а продукты его распада — нефлуоресцирующими. Облучение образцов ультрафиолетовым светом ускоряет распад комплекса.