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Rutacridone as a Fluorescent Dye for the Study of Pollen

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Rutacridone, alkaloid from plant *Ruta graveolens* was used for the histochemical staining of *Hippeastrum hybridum* pollen. The rutacridone fluorescence (excited by UV light at 360–380 nm) has been studied with Karnaukhov-constructed microspectrofluorimeters. Nucleus and DNA were stained by this dye in cells and fluoresced by green light with maxima of 530 and 555 nm, respectively. Development of the pollen was analyzed with this dye.

KEY WORDS: Fluorescence; microspectrofluorimetry; pollen staining; rutacridone.

INTRODUCTION

Analysis of pollen (male gametophyte) development and *in vitro* experiments dealing with fertilization need nucleus staining by fluorescent dyes [1,2]. Acridine orange dye, known as artificial fluorescent probe for nucleic acids, is used for such cellular investigations. The antibacterial agent rutacridone, an alkaloid from *Ruta graveolens*, is chemically similar to acridine orange (Fig. 1). This natural dye also appears to be useful for the cellular studies, in particular for the observation of a nucleus during pollen development. The aim of the paper is to show the possibility of rutacridone as a fluorescent dye for the study of pollen development by microspectrofluorimetry.

EXPERIMENTAL

The object of the study was the male gametophyte pollen of *Hippeastrum hybridum* prepared on the object glass under 20°C and fixed by chloroform or 70% ethanol for 30 min. Rutacridone was isolated, according to Baumert *et al.* [3], from the root of *R. graveolens* and was received from Dr. Kuzovkina. Histochemical staining of pollen (1 mg) or pure preparations (1 mg) of DNA or RNA, bovine serum albumin, the flavonoids rutin and quercetin, or egg lecitin (all reagents from 'Serva', Germany) by rutacridone (1 mg/ml in chloroform) was for

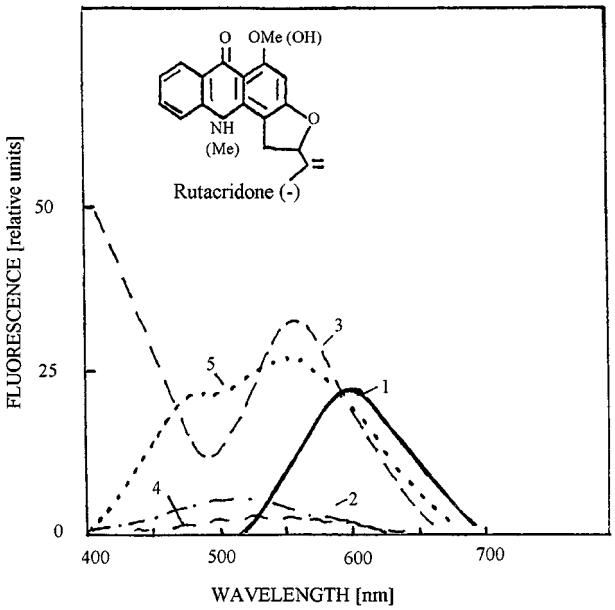

Fig. 1. The fluorescence spectra of the pure rutacridone (1), untreated intact pollen of *Hippeastrum hybridum* (2) and the samples treated with rutacridone (3,4,5) DNA, RNA, and pollen, respectively.

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30 min, after which the samples were washed twice by chloroform at 20°C. After washing in two portions for 10 min, the sample's fluorescence (excited by UV light at 360–380 nm) was analyzed using original-constructed microspectrofluorimeters (1) for the registration of fluorescence spectra [4] and (2) Radical DMF-2 (Radical, Ltd.) interfaced to PC/AT-compatible computer for measurement of the fluorescence intensities at two separate wavelengths [5]. A special program, Microfluor, makes it possible to obtain the distribution histograms of the fluorescence intensities in the red (I_{640}) and green (I_{530}) regions of the spectrum and to perform statistical treatment of the data using the Student *t*-test. To compare the results of the staining by rutacridone with staining by acridine orange, pollen was also treated with a water solution (1 mg/ml) of acridine orange. Pollen germination was studied on the object glasses on wet paper in Petri dishes in nutrient medium—10% sucrose [6].

RESULTS

The staining of dry pollen with rutacridone was studied in a comparison with the staining of the pure substances. As seen in Fig. 1, there were maxima in the fluorescence spectra of pure rutacridone at 590–600 nm (orange region) and of the preparations of pure DNA, stained with rutacridone, at 555 nm, whereas pure RNA and bovine albumin, staining with rutacridone, had very weak or no luminescence in these conditions. Lipids, such as egg lecitin in our case, treated with rutacridone, had no fluorescence in the visible region of the spectra. Among other fluorescing substances of the pollen of *H. hybridum* are flavonoids, such as rutin and quercetin, that themselves did not fluoresce in the green range of spectra, but rather at 580–600 nm [7]. Their crystals, moistened with rutacridone solution, did not change fluorescence color (green fluorescence was not seen). Intact pollen of

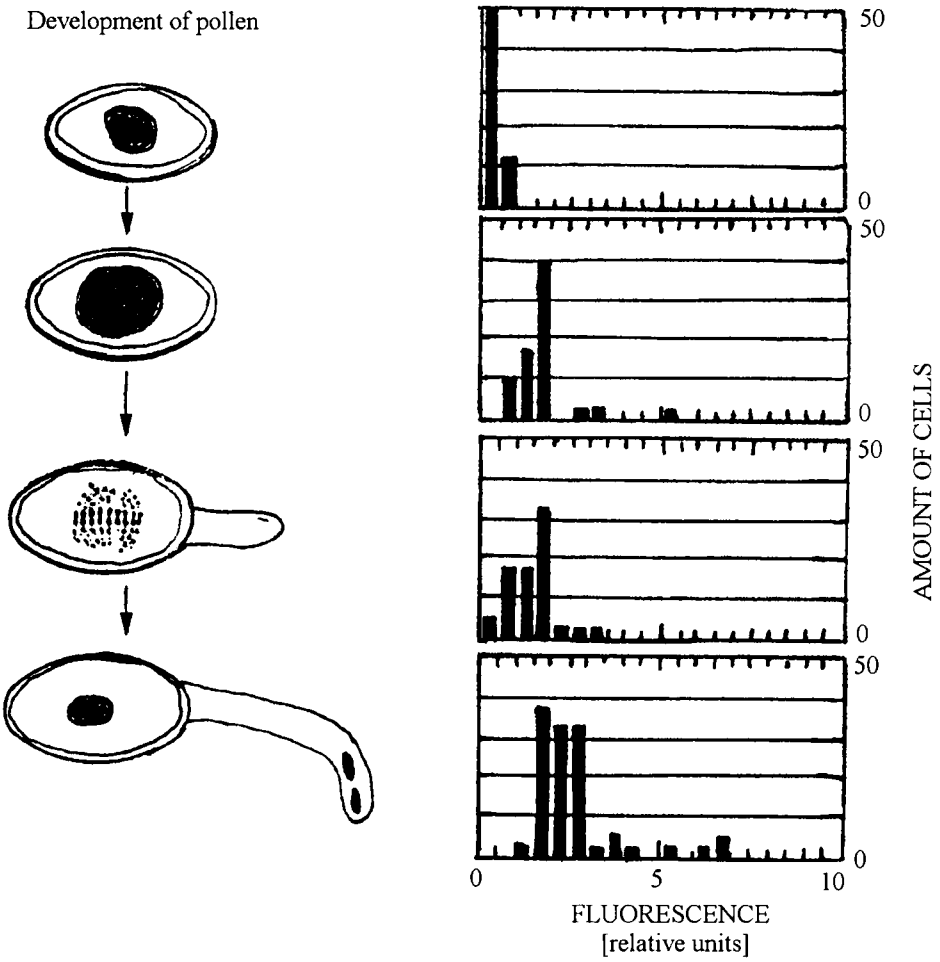

Fig. 2. Summed histograms of the green fluorescence intensity at 530 nm in pollen of *Hippeastrum hybridum* during its development. 0 min: dry pollen; 15, 90, 120 min: time after moistening.

Hippeastrum hybridumi have a weak luminescence at 480–500 nm, but after treatment with rutacridone demonstrated intensive green luminescence at 530–550 nm in the middle of the cell. Other parts of the cell had red emission at 600–630 nm. Thus, green fluorescence in the center of pollen shows that rutacridone binds mainly with the nucleus in cells. We also confirmed this conclusion by experiments with pollen treated with acridine orange. Acridine orange dimers bind to single-stranded nucleic acids (mainly to ribosomal RNA), whereas the band at 530 nm in the green region is associated with its monomers intercalated into double-stranded nucleic acids (DNA, for the most part). Acridine orange stained the pollen nucleus, which green fluoresced, as was seen with rutacridone.

The pollen staining by rutacridone was also used during the study of pollen development (pollen tube formation) after 0, 15, 90, and 120 min after the moistening. Green fluorescence intensity at 530 nm was registrated as histograms (Fig. 2). Dry pollen has one nucleus, and the histogram had unimodal character. Fifteen min after moistening the pollen cell was swollen, and the green intensity of the nucleus grew as well. This is the stage when the mitosis began and new DNA accumulated. At 90 min after moistening, the pollen tube started to develop, and the divergence of chromosomes was well seen as the green lightening strands of spindle. Green-lightening chromosome division has been observed, which confirmed the DNA binding with rutacridone. After 120 min of development, the primary nucleus forms two new nucleuses for the generative cells, named spermia. The formed spermia moved to the tip of the growing pollen tube (if it occurs on the pistil, the one spermium interacts with the egg cell, and so the fertilization takes place). Visually under luminescent microscope and on the histograms three green-lightening nuclei were observed—one of vegetative cell with the pollen tube and two spermia. Our parallel experiments with acridine orange also have shown the presence of two spermial nuclei in the growing pollen tube. Thus, rutacridone may serve as a fluorescent probe for the analysis of the development of pollen grains.

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