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Note

Detection and assay of dihydroergot alkaloids by thin-layer chromatography using *o*-phthalaldehyde–sulphuric acid reagent

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Ergot alkaloids having an ergol-9-ene ring system (ergolenes) exhibit excellent fluorescence properties, which may be measured directly by fluorimetry[2–3]. Their 9,10-dihydro derivatives (ergolines), however, are almost devoid of fluorescence.

In attempts to form fluorogenic derivatives that can be utilized for quantitation, the reaction between *o*-phthalaldehyde and ergolines promised to furnish a satisfactory reaction product. This reagent has been applied earlier, primarily in studies on amino acids[4–8], peptides[8,9], proteins[10], histamine[11–16], spermidine[17], sveeral indole derivatives[18–23] and other compounds of biological interest[24–26]. However, both the composition of the reagent and the reaction conditions were unsatisfactory in the case of ergolines because of insufficient sensitivity and poor stability. In our approach to raise the acidity and acid concentration of the medium, favourable results were obtained at high contents of strong acids. Among numerous variants, optimal sensitivity was achieved if *o*-phthalaldehyde was dissolved in concentrated sulphuric acid. A very light-coloured solution was obtained in this way, which remained stable for several hours at room temperature. On spraying thin-layer chromatograms with this solution, dihydrolysergic acid exhibited a stable blue fluorescence under UV light at long wavelength (366 nm). This reaction seems to be generally applicable in the assay of ergolines.

This paper describes the simultaneous detection and *in situ* quantitation of ergolenes and some corresponding ergolines based on this *o*-phthalaldehyde–sulphuric acid fluorogenic reaction.

EXPERIMENTAL

Support

DC-Alufolien Kieselgel 60 foil (devoid of fluorescent indicator), Type 5553, was obtained from E. Merck (Darmstadt, G.F.R.). Prior to use the foil is developed twice with methanol to remove interfering impurities, and is then heated at 110 °C for 15 min.

Standard materials

d-Lysergic acid (grade II), ergotamine tartrate, dihydroergotamine tartrate

and ergocristine sulphate were obtained from Sigma (St. Louis, MO, U.S.A.). Dihydrolysergic acid and dihydroergocristine were prepared in our laboratory.

Thin-layer chromatography

Methanol solutions were prepared from the above-mentioned compounds (*d*-lysergic acid and dihydrolysergic acid 10 μg/ml; all others 25 μg/ml). These solutions (5 μl each) were spotted on the plate (no air current was used for drying) and developed with ethyl acetate–methanol (65:35). The plates were left to stand in the dark to dry for 5 min (prolonged standing would have resulted in reduced spot intensity), photographed (light mercury lamp, 366-nm filter Camag, Muttenz, Switzerland), then sprayed immediately with the reagent described below. The spots formed are stable, can be photographed (as above) and even scanned after several hours.

Reagent

A solution of *o*-phthalaldehyde (Reanal, Budapest, Hungary) in concentrated sulphuric acid (0.2% w/v) was prepared 5 h prior to application (it can be stored in a refrigerator for 2–3 days without loss of activity).

Quantitative assay

An Opton PMQ II spectrophotometer equipped with a Camag Z-scanner was used, with excitation at 363 nm (filter) and absorption at 470 nm (monochromator). The fluorescence intensity of the spots was recorded with a Philips X–Y recorder

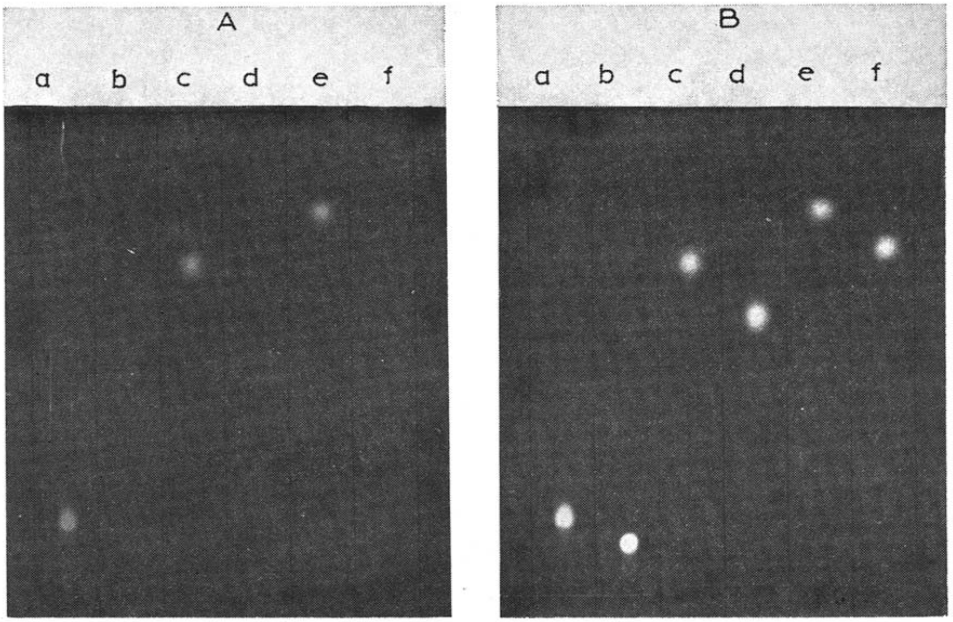

Fig. 1. (A) Photograph taken in UV light (355 nm) prior to spraying (original fluorescence). (a) Lysergic; (b) dihydrolysergic acid; (c) ergotamine; (d) dihydroergotamine; (e) ergocristine; (f) dihydroergocristine. Concentrations: 50 ng (a, b) and 125 ng (c–f). (B) Same chromatogram as in (A) after spraying with *o*-phthalaldehyde–sulphuric acid reagent.

(Type PM 8141). Quantitation was carried out on the basis of the surface integral of the curves obtained.

RESULTS AND DISCUSSION

Fig. 1 shows the fluorescence patterns of several ergolenes and ergolines under UV light, (A) prior to and (B) after spraying with *o*-phthalaldehyde. It is clearly discernable that initially only ergolenes exhibit fluorescence, whereas after spraying the ergolines also show spots of high intensity.

The recorder traces shown in Fig. 2 indicate that the intensity of the ergoline spots even exceeds those of the ergolenes. The fluorogen formed is extremely stable, virtually no change in intensity being detected even after 24 h. The signal-to-noise ratio is highly favourable. The baseline of Fig. 2 is approximately linear.

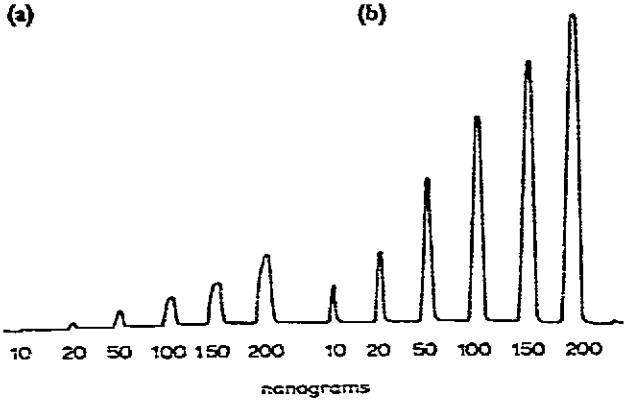

Fig. 2. Scan-recorder trace of (a) lysergic acid and (b) dihydrolysergic acid in the concentration range 10–200 ng.

The method developed permits the detection of 1 ng of ergoline. In the concentration range 10–200 ng the calibration graph was linear and the relative standard deviation was 3.2%.

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