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Note

Quantitative analysis of pteridines in biological extracts by direct fluorometry on thin layers

The separation of pteridines by thin-layer chromatography is widely used[1–3]. Quantitative analysis is usually carried out by the elution of spots and determination of the pteridines in solution. In such determinations it is necessary to determine recovery rates, and the scan cannot always be correlated with the original substance because of the instability of many pteridines.

This paper describes a method for the direct fluorometry of pteridines in biological extracts on thin-layers.

Materials and methods

Pteridines were extracted from a platyfish–swordtail hybrid (*Platypoecilus maculatus* from Rio Jamapa, Mexico, × *Xiphophorus helleri* from Rio Lancetilla, Honduras), in which the expression of the dorsal red gene contributed by *P. maculatus* is enhanced[4]. A 1-g amount of dried skin and fins was homogenized with a glass homogenizer in 20 ml of methanol–pyridine–water (4:1:5) according to the method of DESCIMON AND BARIAL[2], and a volume from the extract equivalent to 1 mg of dried material was subjected to thin-layer chromatography on cellulose (Fertigplatten, Merck, Darmstadt, G.F.R.) with isopropanol–2 % ammonium acetate solution (1:1) containing 0.25 % of mercaptoethanol using Chromatanks (Shandon, London, Great Britain; chamber saturation). The extraction and chromatography were carried out in the dark or under safe-light conditions.

Fluorometry was carried out using a "Dünnschicht-Spektralphotometer" (Zeiss, Oberkochen, G.F.R.) under the experimental conditions given in Table I. Fluorescence units, F (relative units), were recorded with a Servogor instrument (Metrawatt, Nürnberg, G.F.R.). Isoxanthopterin and 9(5)-aminoacridine hydrochloride (both from Fluka, Buchs, Switzerland) were used as standards.

Results

Fig. 1 shows the separation of pteridines on thin layers (UV, 365 nm) and typical curves obtained from scanning the spots in remission using an M 365 filter (transmitting at 365 nm) as a primary filter. The scanning of the spots is carried out according to the decreasing instability of pteridines. In all of the determinations, the fluorescence maxima are identical with the wavelength used on the monochromator except for the drosopterin derivatives, for which the wavelength at 542 nm is used, although the fluorescence maxima are 555, 542 and 543 nm for neodrosopterin, drosopterin and isodrosopterin, respectively. The linearity between the total fluorescence units (peak area) and the amount of material present is reproducible. Linearity is observed over a certain range of concentrations, which differs for different pteridines, *e.g.*, for isoxanthopterin between 0.049 and 0.5 μg (Fig. 2a). Amounts of pteridines of

TABLE I

QUANTITATIVE ANALYSIS OF PTERIDINES BY DIRECT FLUOROMETRY ON THIN LAYERS WITH A "DÜNNSCHICHT-SPEKTRALPHOTOMETER" (ZEISS)

Set up of spectrophotometer: Pr–M (primary filter M 365; thin layer; monochromator); aperture 0.3 × 12.0 mm.

Pteridines	*Sequence of scanning*	*Wavelength on monochromator (nm)*	*Amplification*	*Standard deviation of 7 measurements*	*Relative standard deviation (%)*
Neodrosopterin	Third	542	5/ 1/II/A	± 3.5	± 12.0
Drosopterin	Third	542	5/ 1/II/A	± 3.2	± 4.5
Isodrosopterin	Third	542	5/ 1/II/A	± 1.9	± 4.1
Isoxanthopterin	Fourth	404	7/ 1/ I/A	± 7.7	± 2.5
Sepiapterin	First	515	3/ 1/II/A	± 5.6	± 3.1
Rana-chrome-3	Second	444	1/10/ I/A	± 8.5	± 5.0
Biopterin	Second	444	1/10/ I/A	± 8.5	± 5.0

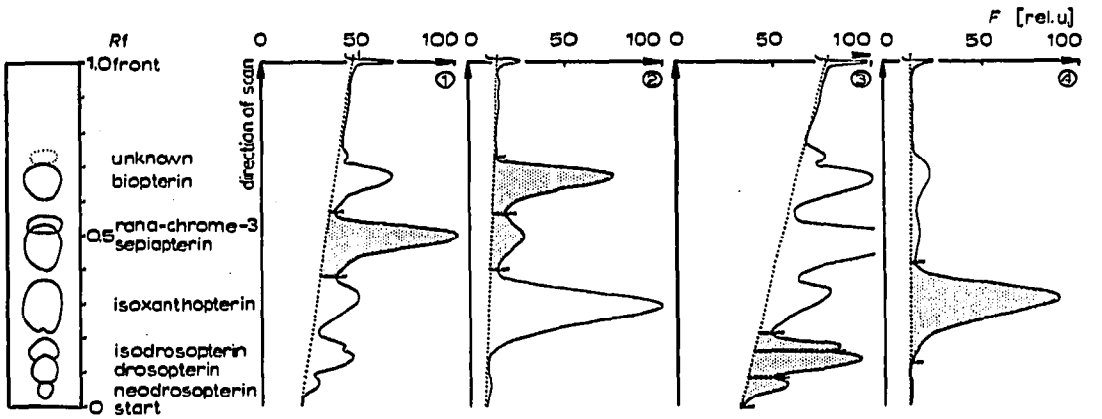

Fig. 1. Separation of pteridines on cellulose layers (left). The R_F values can be calculated by means of the scale given. The pteridines listed correspond both to the location of the spots (left) and to the peaks (right) (for details, see *Materials and methods*). Sequence of scanning: 1 = sepiapterin, 2 = rana-chrome-3 and biopterin, 3 = drosopterins and 4 = isoxanthopterin. The dotted lines were used for the determination of peak areas.

unknown molecular weight, such as the drosopterins, or those with high instability such as sepiapterin and biopterin, are calculated relative to a standard. The drosopterins, sepiapterin and biopterin are determined in fluorescence intensity units of 9(5)-aminoacridine hydrochloride (Fig. 2b). The relative standard deviation for the determination of the individual pteridines is about ± 5 % (Table I).

The method has the advantage of relating fluorescence units of pteridines in a biological extract to a known amount of stable standard under given conditions. The method is rapid, accurate and reproducible, and the recovery calculations that have to be made when making measurements after the elution of spots are not necessary.

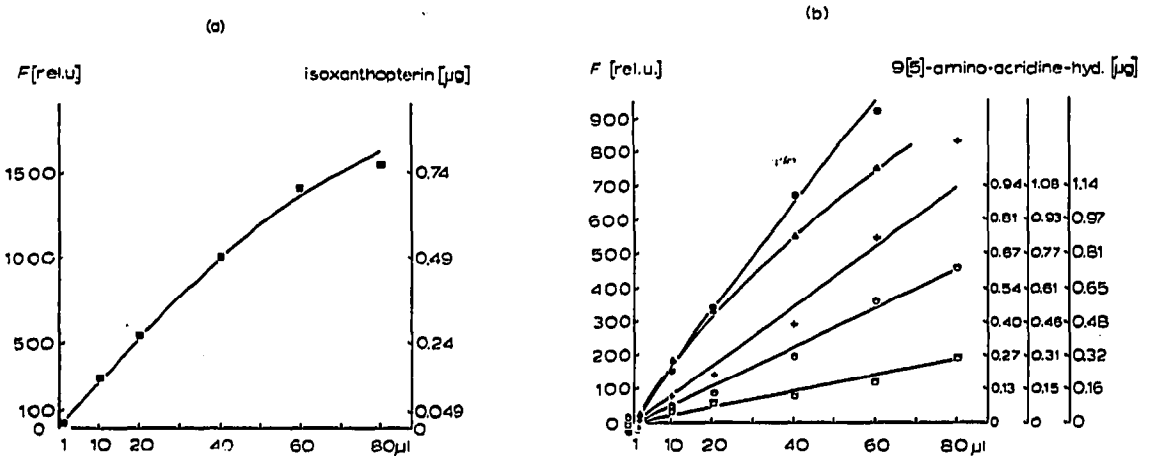

Fig. 2. Relationship between fluorescence units (peak area) and the amount (μg) of materials of (a) isoxanthopterin and (b) 9(5)-aminoacridine hydrochloride present in a certain volume of extract (μl). The volumes given represent the extract as described in the text. (a) Correlation between fluorescence units and volume of extract and pure isoxanthopterin; (b) correlation between fluorescence units and volume of extract and 9(5)-aminoacridine hydrochloride. The amount of standard giving the same F (relative units) as the extract was determined under exactly the same conditions as those for the determinations of the pteridines listed on the left. ■, Isoxanthopterin; □, neodrosopterin; ○, isodrosopterin; +, drosopterin; △, biopterin; ●, sepiapterin.

The method can be used to determine unstable pteridines in their native state and therefore has a greater utility for determining pteridines in biological extracts.

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