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## Note

# Simultaneous separation of pteridines and ommochrome precursors by paper chromatography

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Ommochromes, found in eyes of all arthropods and many molluscs[1], are products of a series of enzymatic reactions involving the amino acids tryptophan (Trp), N-formylkynurenine (Nfk), kynurenine (kyn), and 3-hydroxykynurenine (OHk). Pteridines (drosopterins and closely related compounds) are found in a variety of insects as well as many vertebrates[2]. In this paper we present a simple paper chromatographic method for the simultaneous separation of the ommochrome precursors described above and pteridines. Because two-dimensional development is not required, multiple samples can be chromatographed on the same chromatogram. Our procedure was developed for use with larval, pupal, and adult *Drosophila melanogaster*; however, the method is applicable to other organisms as well.

The chromatographic procedure is divided into two steps: (1) separation of Trp, Nfk, kyn, and OHk from pteridines, as well as separation of individual pteridines and (2) separation of individual ommochrome precursors. The first procedure follows that of Hadorn and Mitchell[3] with some modification. Material used for chromatography is homogenized in acetone–water (1:1), centrifuged at 500 *g* for 30 min, and resulting supernatants are spotted (10 $\mu$l) on sheets of Whatman No. 1 chromatographic paper (10 in. × 10 in.) at 1-in. intervals on a line 1/2 in. from the bottom edge of the paper. A standard containing a mixture of the four amino acids (10 mg/ml) in acetone–water (1:1) is spotted on the two lateral margins. Following spotting, the sheets are air-dried, rolled into cylinders, and placed in 6 in. × 18 in. cylindrical chromatographic jars containing 100 ml of the first developing solution (*n*-propanol–5% ammonia, 2:1). After 3½ h the chromatogram is air-dried, and cut horizontally between the OHk spot and the single spot containing try, Nfk, and kyn. All compounds can be identified with a long-wavelength ultraviolet light. Pteridines and ommochromes are visible under the ultraviolet light either because of their natural fluorescense or fluorescence following interaction with cellulose fibers in the filter paper[4,5]. The top portion of the chromatogram is again rolled into a cylinder and developed in 50 ml of distilled water for 30–35 min. $R_F$ values calculated for each compound in step 1 are as follows: drosopterin (0.09), isoxanthopterin (0.27), sepiapterin (0.45), biopterin and pterin (0.55), OHk (0.59), and Trp, Nfk, and kyn (0.73). $R_F$ values for step 2 are: Trp (0.71), kyn (0.76), and Nfk (0.90).

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Filter combinations used for quantification of pteridines have been reported[6]. Fluorescence emission maxima for all the amino acids (excitation wavelength: 360 nm) are between 464 and 470 nm on Whatman No. 1 chromatographic paper.

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