Separation of riboflavin from sepiapterin by paper chromatography

Presence of brightly colored pteridines in the integument of amphibians suggests that they are associated with pigmentation[1,2]. Identification of these pteridines can be performed by paper chromatography utilizing the following solvent systems: water–saturated butanol, isopropanol–2% ammonium acetate (1:1), *n*-propanol–1% ammonia (2:1), *n*-butanol–acetic acid–water (4:1:1), *n*-propanol–ethyl acetate–water (7:1:2), *n*-butanol–acetic acid–water (4:1:1), 5% acetic acid, 3% ammonium chloride and 3% urea[2].

In amphibian integument some yellow pigmentation is due to riboflavin, sepiapterin, or a mixture of riboflavin with sepiapterin[3,4]. Since both sepiapterin and riboflavin have the same R_F values in the above solvents and have yellow fluorescence, identification of these pigments is difficult. Sepiapterin can be identified by breaking it down to 2-amino-4-hydroxypteridine-6-carboxylic acid in the presence of light[5]. This compound fluoresces blue and has a different R_F value than sepiapterin. Because of the possibility that sepiapterin may not be completely broken down to 2-amino-4-hydroxypterine-6-carboxylic acid resulting in a yellow compound that could either be sepiapterin or riboflavin, a new solvent system was needed to separate these compounds.

FROST[6] found that the solubility of riboflavin could be increased by the addition of borate. ZITTLE[7] believes the effect of borate on riboflavin is that the borate complexes with the ribityl moiety of riboflavin. Because of the increase in solubility, resulting in the increase in partition coefficient of riboflavin, various borate solutions were tested for the stationary phase of the new solvent. It was found that sodium borate in combination with *n*-propanol (1:1) would give satisfactory results in separating riboflavin from sepiapterin. For this reason *n*-propanol–1% sodium borate (1:1) was utilized in the second dimension to separate riboflavin from sepiapterin. With this method sepiapterin has an R_F value of about 0.47 and riboflavin an R_F value of about 0.37. This was verified by using known sepiapterin and known riboflavin.

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