The Pigments of Snake Skins

1. THE ISOLATION OF RIBOFLAVIN AS A PIGMENT OF THE SKINS OF THE GREEN SNAKES *PHILOTHAMNUS SEMIVARIEGATUS* AND *DISPHOLIDUS TYPUS*

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It is frequently noted that when whole snakes or their skins are preserved by immersion in 40% formalin solution, a greenish yellow colour is imparted to the solution, and that this effect is particularly marked with green snakes. A survey of the literature showed that no previous work had been done on snake-skin pigments.

Extraction with absolute ethanol of the skins, freed from organs, of specimens of the green snakes *Philothamnus semivariegatus* and *Dispholidus typus* gave yellow solutions which had a greenish fluorescence in daylight. The amount of pigment obtained was too little for a rigorous, chemical identification, but the general chemical properties of the extract from *P. semivariegatus* suggested that riboflavin was present. Methods for the identification of riboflavin by paper chromatography have been published (Forrest & Todd, 1950; Crammer, 1948), and use on the snake-skin extracts of the developing solvents described by these authors showed that riboflavin was the only pigment present in the extract from *P. semivariegatus*, and that riboflavin and another, unidentified, yellow pigment were present in the extract from *D. typus*. Comparison of the ultraviolet absorption spectra on paper (Bradfield & Flood, 1952) of the yellow pigment from *P. semivariegatus* and of riboflavin showed that they were identical.

The skin of both specimens after extraction was bluish grey and this residual colouring matter could only be obtained by warming with 0·01N sodium hydroxide solution, when the extracted skin turned black. Investigations on this pigment and on the yellow pigment from *D. typus* are proceeding.

EXPERIMENTAL

The organ-free skin of a specimen of *P. semivariegatus* was extracted in the dark with absolute ethanol in a Soxhlet extractor, the solution was then cooled and filtered from insoluble matter. Although no pigment could be isolated from this solution, its yellow colour, strong, green fluorescence in daylight, and the ready loss of both colour and fluorescence on irradiation in sunlight or on addition of dilute acid, dilute alkali, or $NaHSO_3$ suggested that the pigment present was riboflavin.

The organ-free skin of a specimen of *P. semivariegatus* was extracted in the dark with a mixture of water:pyridine:*n*-propanol (1:3:1, v/v throughout) (50 ml.) on a steam bath (45 min.), cooled, and filtered from insoluble material. The solution was yellow with a green fluorescence in daylight. Similar extraction of the skin of a specimen of *D. typus* gave a yellow solution with only a faint green fluorescence in daylight. Paper chromatography of these extracts and of a

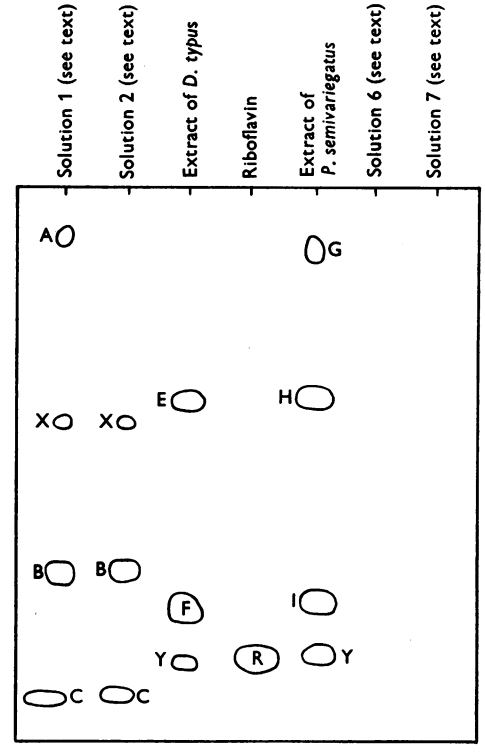

Fig. 1. Paper chromatogram developed with water: pyridine:*n*-propanol (1:3:1, v/v) and solvent allowed to drip off lower edge of paper. Spots A–C, E–I and X, were colourless, showing the following fluorescences in u.v. light: A, G, X, yellow; B, grey; C, bluish grey; E, H, light-blue; F, I, bluish violet. R and Y were yellow in daylight, fluorescing yellow in u.v. light. R and Y (fifth column) faded on exposure to daylight, Y in third column did not. No spots were visible in daylight or in u.v. light in the sixth and seventh columns.

saturated solution of riboflavin in the water:pyridine:*n*-propanol solvent on descending chromatograms of Whatman no. 1 paper irrigated with this solvent (Forrest & Todd, 1950) showed that each extract gave a number of colourless spots which fluoresced in ultraviolet light and a yellow spot which travelled at the same speed as the riboflavin spot.

To show that none of the other substances found in the snake-skin extracts was a decomposition product of riboflavin due to irradiation or chemical treatment of the extract, solutions of authentic riboflavin solutions were treated as follows.

A saturated solution of riboflavin in water:pyridine:*n*-propanol was irradiated in tropical sunlight (4·5 hr.). Half of this solution was retained (solution 1); the remainder was warmed on a steam bath in the dark (45 min.) (solution 2). A saturated solution of riboflavin in distilled water was irradiated in tropical sunshine (4·5 hr.). Pyridine (6 ml.) and *n*-propanol (2 ml.) were added to this solution (2 ml.). Half of it was kept (solution 7); the remainder was warmed on a steam bath in the dark (45 min.) (solution 6).

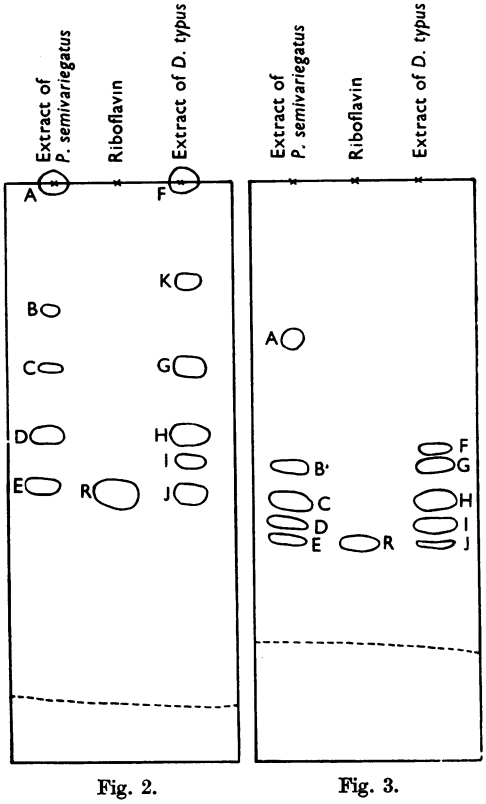

Fig. 2.

Fig. 3.

Fig. 2. Paper chromatogram developed with *n*-butanol: acetic acid:water (Crammer, 1948) for 20 hr. Solvent front marked by dotted line. Spots A–J were colourless, showing the following fluorescences in u.v. light: A, D, F, H, bluish violet; B, yellow; C, G, I, light-blue; E, yellow, becoming greyish yellow; J, yellow, becoming greyish, then light-blue. K was yellow, did not fade in daylight, nor did it fluoresce. R was yellow, fluorescing yellow, and becoming greyish yellow on prolonged u.v. irradiation.

Fig. 3. Paper chromatogram developed with *tert.*-butanol: pyridine:water (50:15:35, v/v) for 34 hr. Solvent front marked by dotted line. Spots A–D, G–I were colourless, showing the following fluorescences in u.v. light: A, yellow; B, D, G, I, light-blue; C, H, bluish violet. E, J and R had the properties of R in Fig. 1, F of spot K in Fig. 2.

Paper chromatography of these four solutions, the two snake-skin extracts, and a saturated solution of riboflavin in water:pyridine:*n*-propanol (0·02 ml. of each solution) by the descending method on Whatman no. 1 paper gave the chromatogram in Fig. 1 which shows that none of the substances obtained from the snake-skin extracts was derived from riboflavin by decomposition (spot G appeared only after the extract had been kept for several days), and that both snake-skin extracts contained a substance, the behaviour of which in the solvent system was identical with that of riboflavin. It was noted that the spot Y in column 3 (extract of *D. typus*) did not lose its yellow colour on exposure to light as did the riboflavin spot. Use of other solvent systems showed that this spot could be resolved into riboflavin and a second, non-fluorescent, yellow pigment.

Paper chromatography of the snake-skin extracts and a saturated solution of riboflavin in water:pyridine:*n*-propanol (0·02 ml. of each solution spotted on paper and air-dried) by the descending method on Whatman no. 1 paper using *n*-butanol:acetic acid:water (Crammer, 1948) and *tert.*-butanol:pyridine:water (50:15:35, v/v; Forrest & Todd, 1950) as developing solvents gave chromatograms, Figs. 2 and 3, which show that the extract of *P. semivariegatus* contains only one yellow pigment, which is identical with riboflavin, and that the extract of *D. typus* contains two yellow pigments, one of which is identical with riboflavin.

A paper chromatogram of the extract of *P. semivariegatus* and a saturated solution of riboflavin in water:pyridine:*n*-propanol was developed with this solvent and the paper was air-dried (1 hr.) and then dried at 100° (45 min.). The riboflavin and yellow-pigment spots were cut off the paper and their ultraviolet absorption spectra were determined on the paper by the method of Bradfield & Flood (1952). The absorption maxima were for the yellow pigment, 260 and 370 mμ.; and for riboflavin, 260 and 360 mμ.

SUMMARY

1. Riboflavin occurs as a yellow pigment in the skins of the green snakes *Philothamnus semivariegatus* and *Dispholidus typus*.

2. Another yellow pigment occurs in the skin of *D. typus*.

3. Both skins also contain a pigment which is extractable by 0·01 N sodium hydroxide solution.

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