SEPARATION AND IDENTIFICATION OF BILE ACIDS IN SOME REPTILES USING THIN LAYER CHROMATOGRAPHY

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SUMMARY: The pattern of secretion of bile acids in different five species of reptiles has been studied using thin layer chromatography. On the basis of R_f value determination and specific color development, it was found that while free bile acids are absent, taurochenodeoxycholic and glycohyodeoxycholic acids are present in all the species studied. The presence of glycocholic acid was characteristic of Cobra alone.

In the present work, attempt has been made to identify the bile acids in some hitherto uninvestigated reptilian species on the basis of their R_f values (1), and specific color development (2,3) after separating them by thin layer chromatography.

MATERIALS AND METHODS

Following species were used for this purpose.

Wall lizard (Hemidactylus flaviviridis)

Garden lizard (Calotes versicolor)

Skink (Mabuya carinata)

Cobra (Naja naja)

Russell's viper (Vipera russellii)

Following solvent phases were prepared by using freshly distilled quality solvents for resolution of free and conjugated bile acids.

(i) Ethyl acetate:glacial acetic acid (96:4, V/V). This phase was used for resolution of free bile acids only.

(ii) Amyl acetate:glacial acetic acid:n-propanol:water (40:30:20:10, V/V). This phase was used for resolution of conjugated bile acids only.

(iii) Propionic acid:isoamyl acetate:water:n-propanol (15:20:5:10, V/V). This phase was used for resolution of both - free and conjugated bile acids.

The degree of resolution of bile acids was determined by spraying with the following freshly prepared detecting agents.

(i) 15 ml concentrated sulphuric acid in 85 ml anhydrous n-butanol.

(ii) 20 g anhydrous antimony trichloride dissolved in 50 ml anhydrous n-butanol and mixed with 10 ml concentrated sulphuric acid and 20 ml glacial acetic acid.

(iii) 5 ml concentrated sulphuric acid in 95 ml acetic anhydride.

(iv) 2 g ferric chloride in 83 ml anhydrous n-butanol and mixed with 15 ml concentrated sulphuric acid.

(v) 10 g phosphomolybdic acid in 100 ml absolute ethanol.

The reptile was anaesthetized by chloroform and dissected. The intact gall bladder was removed from the body by clearing all associated membranes and ducts and then quickly washed in distilled water. Its contents were extracted in n-butanol at room temperature (25^{o}).

Glass plates (210 x 210 mm and 210 x 80 mm) were prepared by soaking successively in the detergent Teepol (Burmah Shell) and concentrated nitric acid, after which they were washed thoroughly first in running tap water and finally with glass distilled water. A suspension of silica gel G (according to Stahl, E. Merck AG, Darmstadt, Germany) was prepared by adding 40 g dry gel to 100 ml distilled water. The suspension was spread on the plates in a layer 250 μ thick. The plates were air dried at room temperature (25^{o}) for 15 min and then activated at 105 ± 1^{o} for 1 h.

The bile acid extract from a species was spotted on the plates using fine capillary. The diameter of the spot did not exceed 3 mm.

Chambers (250 x 250 x 120 mm) were lined with Whatman No. 3 papers and saturated with respective phases.

TABLE I

BILE ACIDS IN DIFFERENT SPECIES

	Conjugated bile acids					Free bile acids
	Tauro-chenodeoxy-cholic acid	Glyco-chenodeoxy-cholic acid	Glycohy-deoxy-cholic acid	Glyco-cholic acid	Tauro-cholic acid	
Wall lizard	+*	+	+	-**	-	-
Garden lizard	+	-	+	-	+	-
Skink	+	-	+	-	+	-
Cobra	+	-	+	+	-	-
Russell's viper	+	+	+	-	+	-

*+ = Present

**- = Absent

The plates were run at room temperature (25°) and the solvent front was permitted to rise 100 mm from the origin.

The chromatoplates were dried at room temperature (25°) for 30 min and then at 100° for about 15 min. After cooling, the plates were sprayed with freshly prepared detecting agents and air dried at room temperature for 15 min. These were then carefully exposed for specific durations at 105 $\pm$ 1° as described by Anthony and Beher (2). After cooling, the colors of the spots were observed in natural light. R_f values were determined for individual spots. These colors as well as R_f values were compared with those of authentic samples.

Identical procedure was followed for all the species mentioned earlier.

RESULTS AND DISCUSSION

The pattern of free and conjugated bile acids in different species as revealed from R_f value determination (1), and color detection (2,3) is indicated in Table I. It is noted that while free bile acids are absent, taurochenodeoxycholic acid and glycohyodeoxycholic acid are present in all the species studied. Glycocholic acid is found to be present only in Cobra (Naja naja).

REFERENCES

1. A. F. Hofmann, J. Lipid Res., 3 (1962) 127.
2. W. L. Anthony and W. T. Beher, J. Chromatog., 13 (1964) 567.
3. S. K. Goswami and C. F. Frey, J. Chromatog., 47 (1970) 126.