the type I, while that in acute and lead poisoning cases is mainly of the type III. In cutanea tarda, the copro has a wider range of the percentage composition, with an average of about 65 % III.

The minimum detectable amount of fluorescent porphyrin esters is 0.002 μg, and 0.1 μg is a convenient quantity to work with. In the case of copro isomers, 0.05–0.1 μg is only barely detectable, because of the spreading spots, and the easy fading of fluorescence on drying.

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Thin-layer chromatography of isatins and corresponding isatin-N-Mannich bases

Recently we have synthesized a series of isatin-N-Mannich bases for pharmacological screening. These results are reported elsewhere[1]. During the course of this investigation it became necessary to develop a suitable method for the separation of isatins from their corresponding Mannich bases. Thin-layer chromatography was chosen because of its rapidity and simplicity. In this communication the results of thin-layer chromatography of these compounds are reported.

Materials

Commercially available isatin, 5-bromoisatin and 5-methylisatin were used.

Isatin-N-Mannich bases were prepared by condensing equimolar proportions of isatin, formaldehyde and appropriate secondary amine[1].

Solvent system

Benzene–ethyl acetate–diethylamine (75:20:5, v/v).

Visualization

Most of the spots were readily visible as such but the optimum visualization was achieved by the use of an ultraviolet lamp.

Method

Pre-coated chromatogram sheets (20 × 20 cm; type K301R, Silica Gel with fluorescent indicator) from Distillation Products Industries were used without activation. Samples were dissolved in acetone and applied to the sheets. The spots were placed on a line 1.5 cm from the lower edge of the sheet at intervals of 2 cm. The

TABLE I

R_F VALUES OF ISATINS AND ISATIN-N-MANNICH BASES

Compound	$R_F \times 100$		
	Run I	*Run II*	*Mean of I and II*
Isatin	44	42	43
N-Morpholinomethylisatin	65	61	63
N-Piperidinomethylisatin	74	67	70.5
N-3-Azabicyclo[3,2,2]nonanomethylisatin	75	67	71
N-3-Azabicyclo[3,2,1]octanomethylisatin	74	68	71
N-Hexamethyleneimino-methylisatin	71	66	68.5
5-Bromoisatin	44	38	41
N-Morpholinomethyl-5-bromoisatin	68	68	68
N-Piperidinomethyl-5-bromoisatin	67	66	66.5
N-3-Azabicyclo[3,2,2]nonanomethyl-5-bromoisatin	71	68	69.5
N-3-Azabicyclo[3,2,1]octanomethyl-5-bromoisatin	67	69	68
5-Methylisatin	38	40	39
N-Piperidinomethyl-5-methylisatin	69	69	69
N-Morpholinomethyl-5-methylisatin	68	73	70.5

rectangular tank was saturated for 2 h and lined with filter paper. The solvent front was 10 cm and the running time was about 20 min.

Discussion

Chromatographic data for three isatins and eleven isatin-N-Mannich bases are summarized in Table I. Although the R_F values of Mannich bases are somewhat close to each other, there is a significant difference when compared to the parent isatin. The method is therefore very useful for the separation and identification of isatins from their corresponding Mannich bases. The method is quite sensitive in that even a trace of isatin, if present in the Mannich base as an impurity, results in the formation of two spots. Thus the method is also useful to ascertain the purity of the Mannich bases.

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