PAPER CHROMATOGRAPHY OF THE ALKALOIDS OF RAUWOLFIA SERPENTINA

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Introduction

The isolation of the alkaloidal complex, serpajmaline,[1] from the fresh undried roots of *Rauwolfia serpentina* has given a fresh impetus to studies in this field in consequence of the fact that this complex, which mainly contains the stronger bases, serpentine, serpentinine, ajmaline and two unknown substances, and is chromatographically free from reserpine, has a strong hypotensive action without any sedative central-depressant action responsible for the undesirable side-effects of reserpine in the treatment of hypertension.[2] Serpajmaline is obtained in the form of a light cream coloured powder, readily soluble in water. In the course of its isolation, a number of other water-insoluble complexes, mainly consisting of the weaker bases, were obtained. For facilitating studies in the composition of these complexes in respect of their individual basic constituents, a need was felt for evolving a suitable technique for their paper chromatographic analysis, which would give a clear picture of the alkaloidal constituents.

While studying the antifertility effects of reserpine and the metabolism of the *Rauwolfia* alkaloids in man and in laboratory animals, Boscott and Kar[3] made use of a number of solvent systems for the separation of these alkaloids by paper chromatography. They have also mentioned some tests for locating these compounds on the paper, but no reference has been made to either the fluorescence or the shape of the spots thus located. Even the R_F values recorded for the following two solvent systems showed overlapping of spots, thus restricting their application to pure samples of the alkaloids, rather than to their mixtures. Their solvent systems were (1) 1% acetic acid in aqueous 5% sodium acetate shaken with *n*-butyl ether, added in small quantities until saturation : the clear aqueous phase was used. (2) Similar to (1) but using tertiary amyl alcohol instead of *n*-butyl ether.

Starting out from the results obtained by these authors, the present investigation was carried out to examine more closely the usability of some of their single phase solvent systems and to devise a solvent which could give a wider range of R_F values and hence a better separation of individual bases in the alkaloidal mixtures.

Method

The following authentic samples of the alkaloids of *Rauwolfia serpentina*, were used in the present investigation : 1. Rescinnamine. 2. Reserpine. 3. Reserpinine. 4. Serpentine. 5. Serpentinine. 6. Ajmalicine. 7. Ajmaline.

The ascending chromatographic technique of Axelrod and Bandurski[4] was employed. A suitable quantity of the alkaloid in chloroform was applied to one corner of Whatman paper No. 1, about 3 cms. from the bottom and side edge. The size of the paper depends upon the dimensions of the bell jar, and 20 × 25 cms. was found suitable in our case. The paper was formed into a cylinder and stapled together with fine threads,

Table 1.—Comparative Efficiencies (in Terms of R_F Values) of Whatman Paper Nos. 1 and 542.

Solvent : Aqueous layer obtained by saturating 10% acetic acid (v/v) in 5% sodium acetate with *n*-butyl ether.

Type of paper	Rescinnamine	Reserpine	Reserpinine	Serpentine	Serpentinine	Ajmalicine	Ajmaline
Whatman No. 542 ..	0.26	0.34	—	0.50	—	—	0.76
Whatman No. 1 ..	0.29	0.39	0.54	0.50	0.37	0.57	0.78

avoiding contact of the adjacent edges, and then suspended by a hook inside the bell jar, placed on a well-greased glass sheet supporting a petri dish containing the solvent. By lowering the hook, the paper was dipped in the solvent system, and the chromatogram was allowed to run until the solvent almost reached the upper edge of the paper, about five hours being required for this purpose. The paper was then thoroughly dried in the air at room temperature and viewed under ultra-violet light.

Experimental

Preliminary experiments were conducted to ascertain the efficiency of Whatman paper No. 1. Using the same solvent system, the R_F values on No. 1 were compared with those obtained by Boscott and Kar on Whatman paper No. 542. From Table I, it seems reasonable to conclude that the two papers are at par with each other.

The location of *Rauwolfia* alkaloids on the paper presented considerable difficulties. It has been reported that when the alcoholic layer, obtained by shaking butanol, hydrochloric acid and water, was taken as the developing solvent, ajmaline took two weeks to become visible under ultra-violet light.[5] In the course of efforts for devising tests to locate the *Rauwolfia* alkaloids on paper, it was observed that ajmaline could be readily detected in ultra-violet light after the paper has been sprayed with a 5% solution of sodium acetate.* Table 2 hows the data obtained on the shape and fluoresence of the spots for the *Rauwolfia* alkaloids.

TABLE 2.—THE SHAPE AND FLUORESCENCE OF RAUWOLFIA ALKALOIDS SEPARATED ON WHATMAN PAPER NO. 1.

Alkaloids	Shape of spots after development	Fluorescence
Rescinnamine ..	Streak	Light green
Reserpine ..	Elongated	Light green
Reserpinine ..	Oval	Bright green
Serpentine ..	Oval	Sky blue
Serpentinine ..	Streak	Violet
Ajmalicine ..	Oval	Light blue
Ajmaline ..	Round	Violet changing to light blue.

After a few trials it was observed that the addition of methanol to the above mentioned solvent system showed a marked effect on the R_F values of all the standard alkaloids. This addition gave a solvent system that could give a clean separation of the alkaloidal mixtures as shown by Table 3. The addition of methanol, in some way, also affects the rate of capillary ascent, thereby reducing the time of the development of the chromatogram. The solvent was prepared in the following manner :—

Acetic acid 1% (v/v) in aqueous 5% sodium acetate is shaken with isobutyl methyl ketone,

* Since the solvent suggested by Boscott and Kar incorporated sodium acetate buffer, the difficulty of locating ajamaline was not encountered by them.

TABLE 3.—THE R_F VALUES, SHAPE AND FLUORESCENCE OF THE RAUWOLFIA ALKALOIDS AFTER DEVELOPMENT WITH THE NEW SOLVENT.

Alkaloids	R_F Values	Shape	Fluorescence
Rescinnamine ..	0.12	Oval	Light green
Reserpine ..	0.22	Oval	Light green
Reserpinine ..	0.40	Round	Bright green
Serpentine ..	0.33	Round	Sky blue
Serpentinine† ..	—	Elongated	Violet blue
Ajmalicine ..	0.49	Round	Light blue
Ajmaline ..	0.74	Round	Violet changing to Light blue.

†R_F Value omitted due to the elongated nature of the spot.

added in small quantities until saturation. To 100 cc. of the clear aqueous phase is added 5 cc. methanol, which is then used for development. A typical composite chromatogram made in this way is shown in Fig. 1, and the measured R_F values together with other data are given in Table 3.

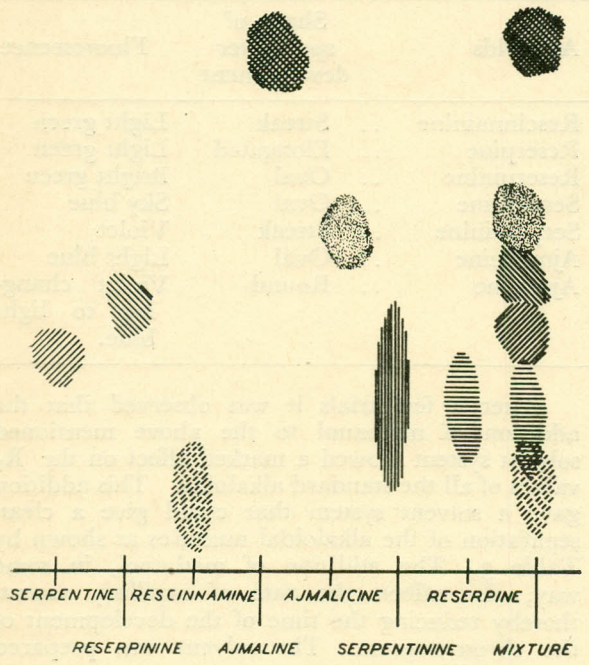

Fig.—A composite chromatogram (6/13 size) showing separation of Rauwolfia alkaloids.

Discussion

The addition of methanol to the solvent seems to affect the movement of all the bases in such a way that a wider range of R_F values is obtained, and the spots become round and compact. This has resulted in a better and clearer separation of the alkaloidal mixtures.

The fluorescence behaviour of ajmaline is somewhat peculiar. It was noted that the authentic samples of ajmaline gave a violet fluorescence which finally changed to light blue, the change being very gradual, so that more than 12 hours are required for the attainment of the final light blue colour. In natural extracts of *Rauwolfia serpentina*, *e.g.*, serpajmaline, on the other hand, ajmaline always gave the typical light blue fluorescence, no violet tinge being noticed. Further work relating to studies in this interesting behaviour of ajmaline is in hand.

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References

1. S. Siddiqui, Pakistan J. Sci. Ind. Research, **1**, 3 (1958).
2. R. Deininger, ibid., **1**, 6 (1958).
3. R.J. Boscott and A.B. Kar, Nature, **176**, 1078 (1955).
4. R.S. Bandurski and B. Aexelrod, J. Biol. Chem., **193**, 405 (1951).
5. P.P. Pillay, S.B. Rao and D.S. Rao, Indian J. Pharm., **17**, 98 (1955).