THE DISTRIBUTION OF ALKALOIDS IN *RAUWOLFIA CAFFRA* SOND. AND RELATED SPECIES

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In its content of reserpine, *Rauwolfia caffra* root-bark compares favourably with other species. The highest proportion of reserpine is found in the inner phloem whereas the ajmaline group of alkaloids, except for their absence in the cork, are more evenly distributed. The alkaloid content of thin bark exceeds that of thick bark and is very low in the wood of stem and root.

In a previous communication[1] the structure of the root and stem of *Rauwolfia caffra* Sond. has been described. The root is known to contain rauwolfine[2], ajmaline[3] and reserpine[3,4] but little is known of the distribution of the alkaloids within the plant or of the relative proportions of reserpine to other bases. To clarify this situation, the assays reported below were undertaken and the results compared with similar analyses of related species.

Experimental

Plant Materials and Extraction of Alkaloids

Four samples of *R. caffra* were investigated. Two were collected by D. B. Fanshawe, Esq., Ndola River, Northern Rhodesia; these comprised thick bark (0·5 cm.) from large roots of unspecified size and smaller whole roots and stems. Another sample was supplied by John Ronaldson Ltd., London and the fourth was collected by S. N. Wimbush, Esq., Conservator of Forests, Northern Nigeria, as *R. welwitschii* Stapf. (now regarded as synonymous with *R. caffra* Sond.). Dr. P. J. Greenway, Nairobi procured the sample of *R. mombasiana* Stapf. and Dr. W. S. S. Ladell the Nigerian *R. vomitoria* Afz. The remaining samples were donated by Riker Laboratories Ltd., England and Riker Laboratories Inc., U.S.A.

A number of root segments of the diameters specified in Table I were selected from each sample, the barks and woods separated and the fractions powdered. For a more detailed analysis of the thick bark of *R. caffra* from Northern Rhodesia, pieces were dissected into five histological portions (Fig. 1) and the corresponding fractions combined to form bulk samples. The cork (1) and the inner functional phloem (5) were easily separated by scraping and the remaining tissues, all containing stone cells, were divided into three portions corresponding approximately to the phelloderm (2) and the largely non-functional outer and middle phloem, (3) and (4).

For the extraction of the alkaloids, 1 g. of powdered sample was intimately mixed with 0·2 g. of calcium hydroxide, moistened with water and allowed to stand overnight. The alkaloids were removed in ether by continuous extraction for 6 hours, *ad hoc* experiments having shown

that under these conditions the extraction of the alkaloids was complete. The residue obtained by removal of the solvent was treated with 2 ml. 1·0N hydrochloric acid and shaken with three separate portions of chloroform. The combined chloroformic extracts were washed successively with 0·13N sodium bicarbonate solution and water and evaporated to dryness under reduced pressure. The residue, protected from light, was used for the determination of reserpine and related alkaloids.

The bases in the acid solution and combined washings were liberated with ammonia, collected in chloroform and washed with water. Evaporation of the solvent furnished a residue containing the more strongly basic alkaloids of the sample, referred to below as the ajmaline group.

Paper chromatography was used to follow the separation process; an amyl alcohol, light petroleum, glacial acetic acid, water mixture (3:1:3:3) proved satisfactory for the development of the chromatograms, which on drying were observed in filtered ultra-violet light. The alkaloids reserpine, rescinnamine, ajmaline and serpentine were used as reference standards. Reserpine, after treatment with acetic acid, gives on irradiation with ultra-violet light, an apple-green fluorescence and similar fluorescent spots of different R_F values were obtained on paper chromatograms from some ajmaline fractions. The small amount of blue fluorescent contaminants in the reserpine fractions could be eliminated in the quantitative assay by the use of a suitable filter and by the enhancement of the reserpine fluorescence as described below. Other authors have also drawn attention to the fluorescent contaminants in the assay of *R. serpentina* root[5–7].

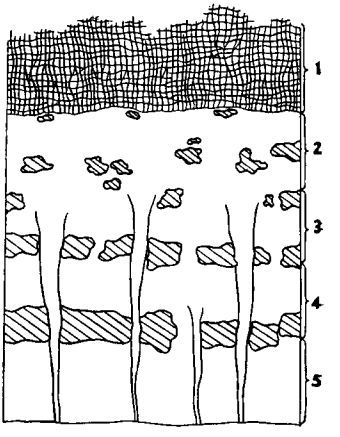

FIG. 1. *Rauwolfia caffra Sond.* General diagram of transverse section of root-bark × 10. 1, cork; 2, phelloderm; 3, outer phloem; 4, middle phloem; 5, inner phloem. Shaded areas of 2, 3 and 4 indicate stone-cell groups.

Estimation of the Alkaloids

The assay of the dried reserpine fractions was based on Dechene's method[8] which in the range of 0·4 to 1·8 μg. reserpine per ml. shows an accuracy of +3·2 to −1·0 per cent. Each residue was dissolved in 5N acetic acid and diluted to 100 ml. Several aliquots of each solution, varying from 0·5 ml. for bark samples to 40 ml. for wood samples, were mixed with 3 ml. of 3 per cent hydrogen peroxide solution and the volumes adjusted to 50 ml. with 5N acetic acid. The solutions were heated in a boiling water bath for 45 minutes, cooled to room temperature and the volume readjusted with 5N acetic acid. The fluorescence of the solution was recorded in a modified Hilger fluorimeter using as primary and

secondary filters, Chance OX1 and OG2 filters respectively. The concentration of reserpine in each solution was obtained by reference to a standard curve using those drum readings from aliquots which corresponded to the same, most suitable, region of the curve. The standard curve was prepared by the use of standard reserpine solutions, of the

TABLE I

Sample*	Alkaloids, per cent**			
	Reserpine Group		Ajmaline Group	
Rauwolfia caffra				
Sample 1. N. Rhodesia				
Bark 0·5 cm. thick ..	0·120 ± 0·004 (3)		0·303 ± 0·002 (4)	
Ditto dissected as Fig. 1				
Cork	0·009 ± 0·001 (3)		0·000	
Phelloderm	0·053 ± 0·002 (5)		0·344 ± 0·001 (5)	
Outer phloem	0·153 ± 0·007 (3)		0·344 ± 0·001 (6)	
Middle phloem	0·157 ± 0·003 (3)		0·278 ± 0·000 (7)	
Inner phloem	0·185 ± 0·003 (4)		0·277 ± 0·002 (4)	
	Bark	Wood	Bark	Wood
Sample 2. Bulked roots (2·7 cm.)	0·550 ± 0·012 (3)	0·011 ± 0·002 (3)	0·926 ± 0·002 (3)	0·009 ± 0·001 (4)
Sample 3. N. Rhodesia Bulked roots (12·0 cm.)				
Bark 0·4 cm. thick ..	0·098 ± 0·009 (3)	0·008 ± 0·000 (3)	0·292 ± 0·001 (8)	0·031 ± 0·002 (4)
Stems	†	0·007 ± 0·001 (3)	trace	trace
Sample 4. N. Nigeria (supplied as *R. welwitschii*)				
Bulked roots (2·0 cm.) ..	0·187 ± 0·007 (3)	0·017 ± 0·001 (3)	0·413 ± 0·002 (3)	0·031 ± 0·001 (3)
Stem bases (6·0 cm.) ..	0·005 ± 0·000 (3)	0·006 ± 0·001 (3)	0·004 ± 0·000 (3)	trace
R. vomitoria				
Sample 1. Nigeria				
Bulked roots (1·0 cm.) ..	0·410 ± 0·006 (3)	0·040 ± 0·003 (3)	0·618 ± 0·003 (4)	0·041 ± 0·002 (3)
,, ,, (2·0 cm.) ..	0·420 ± 0·000 (3)	0·053 ± 0·001 (3)	0·587 ± 0·001 (4)	0·030 ± 0·002 (3)
Sample 2. Commercial sample				
Bulked roots (1·0 cm.) ..	0·648 ± 0·008 (4)	0·037 ± 0·001 (3)	0·704 ± 0·002 (3)	0·034 ± 0·001 (3)
,, ,, (2·0 cm.) ..	0·670 ± 0·004 (4)	0·052 ± 0·002 (2)	0·621 ± 0·003 (3)	0·032 ± 0·000 (3)
R. mombasiana. Kenya				
Bulked roots (2·5 cm.) ..	0·537 ± 0·003 (3)	0·030 ± 0·005 (3)	1·181 ± 0·002 (3)	0·038 ± 0·002 (3)
R. serpentina. India				
Sample 1.				
Bulked roots (1·0 cm.) ..	0·150 ± 0·000 (4)	0·017 ± 0·002 (3)	1·875 ± 0·009 (3)	0·062 ± 0·002 (4)
,, ,, (2·0 cm.) ..	0·120 ± 0·006 (3)	0·014 ± 0·001 (3)	1·613 ± 0·003 (3)	0·031 ± 0·001 (3)
Sample 2.				
Bulked roots (1·0 cm.) ..	0·100 ± 0·006 (3)	0·027 ± 0·000 (3)	0·335 ± 0·002 (4)	0·060 ± 0·000 (3)
,, ,, (2·0 cm.) ..	0·095 ± 0·005 (2)	0·019 ± 0·002 (3)	0·324 ± 0·002 (4)	0·029 ± 0·001 (3)
R. micrantha				
Bulked roots (1·0 cm.) ..	0·143 ± 0·007 (3)	0·032 ± 0·002 (3)	0·388 ± 0·003 (3)	0·025 ± 0·001 (3)
,, ,, (2·0 cm.) ..	0·057 ± 0·003 (3)	0·019 ± 0·001 (3)	0·177 ± 0·001 (3)	0·016 ± 0·000 (3)
R. tetraphylla (*R. canescens L.*)				
Bulked roots (1·0 cm.) ..	0·160 ± 0·000 (2)	0·028 ± 0·004 (3)	0·965 ± 0·006 (3)	0·039 ± 0·000 (3)
,, ,, (2·0 cm.) ..	0·163 ± 0·003 (2)	0·031 ± 0·001 (3)	1·008 ± 0·008 (3)	0·037 ± 0·001 (2)

* Measurements refer to diameter of member except where stated otherwise.
** Arithmetic means ± the standard error with number of assays in parenthesis.
† Assay unreliable—see text.

above concentration range, treated with acetic acid and hydrogen peroxide. Repeated checks of the standard curve throughout the investigation indicated no changes due to instrument variability.

For the determination of the ajmaline group of alkaloids, the solid extract was dissolved in ether and a little chloroform if necessary, and a solution of bromocresol green indicator neutralised to pH 4·5 added. With constant mixing of the two layers, the system was titrated with

0·0025N sulphuric acid to match a prepared end point of pH 4·5. For convenience the alkaloid content was calculated in terms of ajmaline although for some species this may not be the major constituent.

Results and Discussion

The results of assays are given in Table I. For *R. vomitoria* a total alkaloid content of 1·04 per cent has been recorded, of which 10 per cent is reserpine[9]. Roots of small diameter contain a higher percentage of reserpine than the larger ones[10] but no separate analyses for bark and wood are recorded. The results reported here suggest that with *R. vomitoria, R. serpentina* and *R. tetraphylla* the barks of roots of diameter 1 cm. and 2 cm. from any one sample of a species do not differ appreciably in their alkaloid content. Due however, to the relatively higher proportion of wood to bark in the larger roots, the alkaloid content for the 2 cm. whole root would be less than that for the 1 cm. root. As with the other African species *R. vomitoria* and *R. mombasiana*, the root-bark of *R. caffra* contains commercially workable quantities of reserpine and, with barks varying considerably in thickness, a relation between size and alkaloidal content is evident. Should this species be used commercially, the root-wood, which contains little alkaloid, could be rejected at the time of collection. The relative proportions of alkaloids in the root-wood and root-bark appear in most instances to be similar for all the species examined.

With the sample of *R. caffra* bark examined in detail, the alkaloid mixture is not evenly distributed throughout the root-bark (Fig. 1). The cork contains practically no alkaloids. Reserpine is found in highest concentration in the inner phloem with much less in the phelloderm, whereas the ajmaline group of alkaloids are more uniformly distributed with highest proportions in the phelloderm and outer phloem.

It was not possible to make a successful determination of the reserpine content of the stem-bark owing to the presence of an interfering fluorescent principle in the appropriate fraction. Paper chromatograms indicated the possible presence of a small quantity of reserpine and in view of references[9,11] to the use of the stem-bark by native tribes an attempt was made to isolate the weakly basic constituents from 50 g. of bark originating from Northern Rhodesia. No crystalline alkaloid could be isolated; a similar negative result had previously been reported by Schüler and Warren with material from Natal[3].

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