

# VARIATION IN THE CHEMICAL COMPOSITION OF INDIAN SAMPLES OF *CENTELLA ASIATICA*

P. S. RAO AND T. R. SESHADRI\*

*Department of Chemistry, University of Delhi, Delhi-7*

IN a programme of investigation of Indian vegetable drugs there was need to analyse samples of *C. asiatica* obtained from various parts of our country.

*Centella asiatica* (*Hydrocotyle asiatica*) is commonly called Brahmi and Mandukaparni and is well known for its medicinal value<sup>1</sup>; it has therefore been studied by various workers<sup>2-14</sup>. Their results are summarised in Table I.

test) besides free sapogenins and chlorophyll. It was evaporated to dryness, the residue dissolved in minimum quantity of ethyl acetate and light petroleum ether gradually added to it. After the initial separation of a dark green gummy residue, the supernatant clear solution on treatment with excess of light petroleum gave an yellow solid which on TLC (silica gel; toluene: pyridine: acetic acid 10:1:1)<sup>15</sup> showed two bright yellow

TABLE I  
*Chemical components of Centella asiatica Urb.*

Sl. No.	Origin of the plant	Saponin (s)	Sapogenin (s)	Sugars from the saponin (s)	Other components	Ref.
1	Madagascar	Asiaticoside	Asiatic acid	Glucose, Rhamnose		4, 5, 6
2	"	Madecassoside	Madecassic acid	Glucose, Rhamnose		11, 12
3	Ceylon	Centelloside	Centellic acid	Glucose, Fructose	(a) Centic acid (b) Centoic acid	7
4	India		Indocentoic acid			7
5	"	(a) Brahmoside	Brahmic acid	Glucose, Rhamnose, Arabinose	(a) Isobrahmic acid	
		(b) Brahminoside	"		(b) Betulic acid	9, 15
		(c) Asiaticoside	Asiatic acid	Glucose, Rhamnose	(c) Stigmasterol	
6	"	(a) Thankuniside	Thankunic acid	Glucose, Rhamnose	Asiatic acid	8, 14
		(b) Isothankuniside	Isothankunic acid			

Because of the divergence in the results which may be due to the place of origin of the material, to the different techniques involved in the isolation of the components or to the difference in varieties of the plant, we wished to investigate samples from various parts of India using the same method. The present communication deals with our results with samples collected from Hardwar, Dehradun, Jammu, Trivandrum, Madras, Hyderabad and Lucknow. The general method of extraction is as follows. The alcoholic extract was concentrated and diluted with water and extracted successively with light petroleum, ether, ethyl acetate and *n*-butanol. The final aqueous solution was tested for free sugars by paper chromatography.

The light petroleum extract on chromatography on alumina yielded varying amounts of wax, carotenoids and chlorophyll. The ether extract was found to contain flavonoids (positive magnesium-hydrochloric acid colour

spots. Column chromatography of the mixture on silica gel yielded two yellow compounds identical with kaempferol and quercetin. A third flavonoid having a lower *R<sub>f</sub>* value but not identical with myricetin, was found to be present in the mother liquors after the separation of kaempferol and quercetin. These flavonoids were present in all the samples examined.

The ethyl acetate extract was evaporated to dryness, the residue taken up in methanol and fractionated into the neutral and basic lead salts. The lead salts were decomposed with H<sub>2</sub>S in alcoholic suspension when reddish-brown gummy solids were obtained. Both the fractions gave a positive test for the presence of flavonoids and a positive Molisch test. Repeated attempts to crystallise these fractions or to separate the mixture by paper chromatography in different solvents met with little success. On hydrolysis with 7% aqueous sulphuric acid, both the fractions gave quercetin and kaempferol as aglycones and glucose and rhamnose as sugar components.

\* Paper presented at the 19th session of the Indian Pharmaceutical Congress held at Hyderabad, December, 1967.

The *n*-butanol extract was concentrated under reduced pressure. The residue was dissolved in methanol and the solution poured with stirring into excess of dry ether when a colourless solid separated. It was quickly filtered and dried in a vacuum desiccator. The pale brown powdery solid answered tests for saponins. The yields of the saponins from different samples are given in Table II.

TABLE II  
Saponin content of the various samples of *C. asiatica*

Sample No.	Source	Saponin content % of air dry weight of plant
1	Hardwar	3.4
2	Dehradun	3.2
3	Jammu	8.0
4	Trivandrum	1.6
5	Madras	2.3
6	Hyderabad	1.1
7	Lucknow	2.2

The crude saponin mixtures were purified by repeated precipitation from a methanol solution by ether. After ascertaining the absence of free sugars in them, they were examined by TLC (silica gel; *n*-butanol; ethyl acetate: water, 4:1:5, upper phase Fig. 1). Saponin mixtures from sample numbers 1, 2, 3, 4, 6 behaved identically showing

0.58. The absence of brahmoside in samples 1, 2, 3, 4 and 6 is therefore at once discernible. Madecassoside (II) and brahminoside have the same  $R_f$  value but the latter contains arabinose. Hence a method of ascertaining

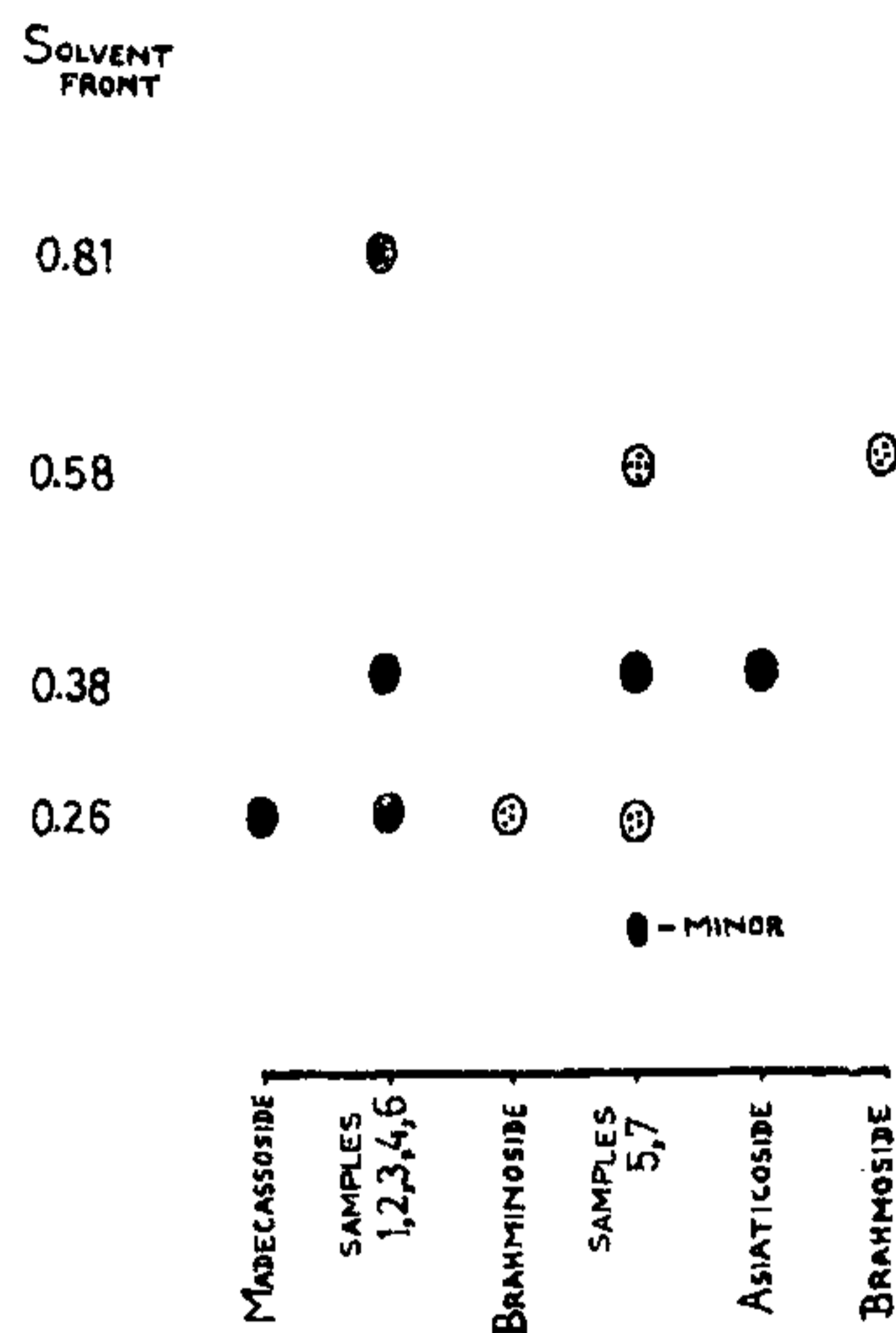
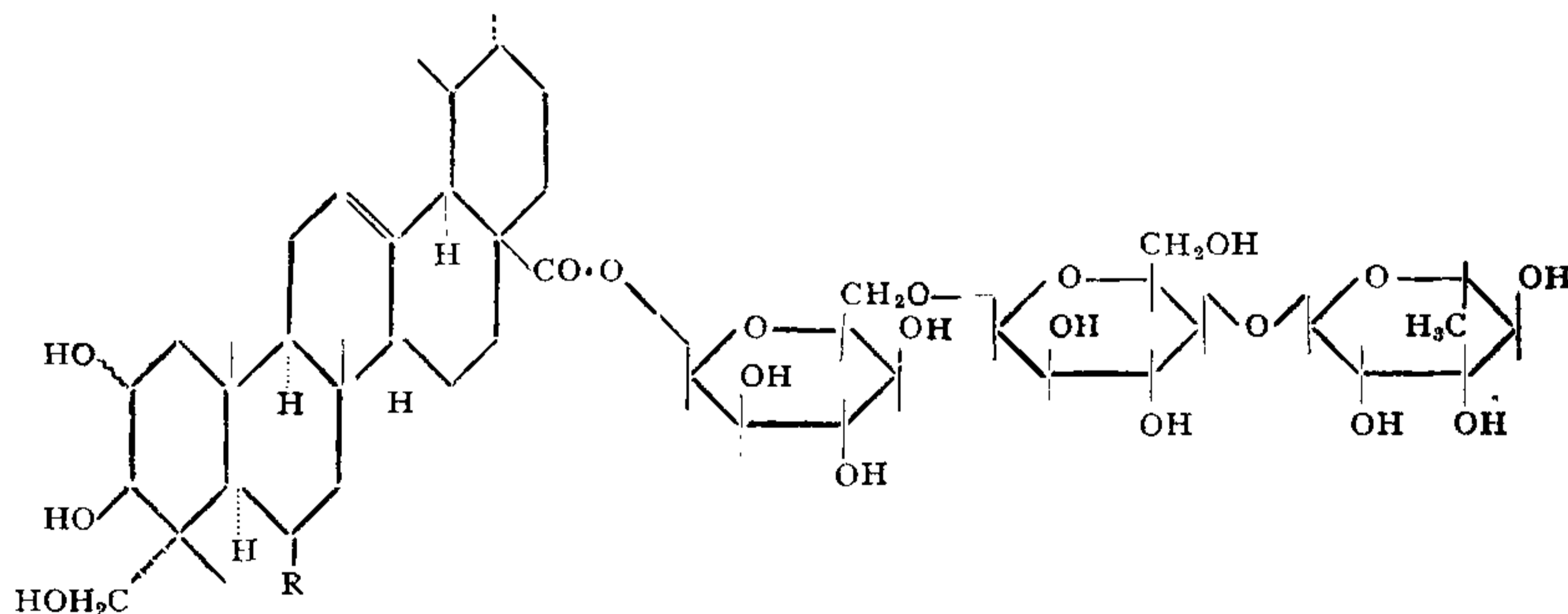


FIG. 1. TLC of saponin mixtures



- I. Asiaticoside,  $R=H$   
II. Madecassoside,  $R=\beta-OH$

three spots with  $R_f$  values 0.26, 0.38 and 0.81 while samples 5 and 7 showed three strong spots with  $R_f$  0.26, 0.38 and 0.58. Authentic samples of madecassoside (II) and asiaticoside (I) had  $R_f$  values 0.26 and 0.38 respectively. Brahminoside and Brahmoside based on the sapogenin brahmie acid ( $\equiv$  madecassic acid<sup>17</sup>) had  $R_f$  values 0.26 and

the presence of the latter is to subject the mixture to acid hydrolysis and test the acid hydrolysate for the presence of arabinose.<sup>18</sup> Tested this way arabinose was found to be absent in the acid hydrolysates of the saponins from samples, 1, 2, 3, 4 and 6. Therefore, brahmoside and brahminoside were absent in these samples and they contain madecassoside

and asiaticoside and a faster moving saponin. Arabinose was present in the samples 5 and 7 which contain brahmoside and brahminoside besides asiaticoside<sup>14</sup> as the saponin constituents.

Hydrolysis of the various saponin mixtures with alkali yielded madecassic and asiatic acids thus confirming that the saponins were of ester type. Acid hydrolysis of the saponin mixtures however gave besides the above, one more compound whose methyl ester agreed with the physical constants of anhydromethyl madecassate reported by Pinhas *et al.*<sup>12</sup> We could not however detect the presence of indocentoic acid,<sup>7</sup> thankuniside and isothankuniside<sup>8</sup> in our samples.

The above results indicate large variations in the yield of saponins depending on habitat; this is fairly common in plant drugs. The sample from Jammu was the richest. Considering the nature of the saponins there seems to be two varieties; the more common one contains asiaticoside and madecassoside whereas the less common one is characterised by the additional presence of arabinose in the saponins, thus forming brahmoside and brahminoside. The sapogenins are the same in both and similarly are the flavonoid components.

#### ACKNOWLEDGEMENTS

Our thanks are due to Dr. C. Dwarakanath for procuring various samples of plant material, Shri V. P. Mahajan, for a sample of material from Jammu area, Dr. H. Pinhas for samples of asiaticoside, madecassoside, methyl asiatate

and methyl madecassate and for comparing our sapogenin methyl ester with methyl madecassate, Dr. R. P. Rastogi for samples of brahmoside, brahminoside and methyl brahmate, and the Indian Council of Medical Research for financial assistance.

1. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants*, Lalit Mohan Basu, Allahabad, 1933, 2, 1193.
2. Bontems, J., *Bull. Sci. Pharmacol.*, 1941, 49, 186.
3. Frierejacque, *Bull. Soc. Chem. Biol.*, 1949, 31, 1510.
4. Boiteau, P., Buzas, A., Lederer, E. and Polonsky, J., *Nature*, 1949, 163, 258.
5. Polonsky, J., *Bull. Soc. Chim. France*, 1952, 649, 1015, 1953, 173.
6. —, Sach, E. and Lederer, E., *Ibid.*, 1959, p. 880.
7. Bhattacharya, S. C., *J. Ind. Chem. Soc.*, 1956, 33, 579, 638 and 894.
8. Datta, T. and Basu, U. P., *J. Sci. Industr. Res.*, India, 1962, 21 B, 239.
9. Rastogi, R. P., Sarkar, B. and Dhar, M. L., *Ibid.*, 1960, 19 B, 252.
10. — and Dhar, M. L., *Ind. J. Chem.*, 1963, 1, 1888.
11. Pinhas, H. and Bondiou, J. C., *Bull. Soc. Chim. France*, 1967, p. 1888.
12. —, Billet, D., Heitz, S. and Chaigneau, M., *Ibid.*, 1967, p. 1890.
13. Rastogi, R. P. and Singh, B., communication *Phytochemistry*.
14. Dutta, T. and Basu, U. P., *Ind. J. Chem.*, 1967, 5, 586.
15. Rastogi, R. P. (Private communication).
16. Natarajan, S., Murthi, V. V. S. and Seshadri, T. R., *Ind. J. Chem.*, 1968, 6, 549.
17. Our sapogenin methyl ester was compared with methyl brahmate as well as methyl madecassate by Drs. Rastogi, R. P. and Pinhas, H. and were found to be identical.
18. "Paper chromatography on Whatman No. 1 filter-paper; *n*-butanol : pyridine : water, 6 : 4 : 3; Aniline hydrogen phthalate spray."

## RANGE OF STRUCTURAL AND ONTOGENETIC STOMATAL VARIATIONS IN THREE SPECIES OF *OCIMUM* (LABIATAE)

N. RAMAYYA AND V. JAGANNATHA RAO

Plant Anatomy and Taxonomy Laboratory, Department of Botany, Osmania University,  
Hyderabad-7, A.P.

**T**HOUGH several workers<sup>2-4,6</sup> have reported structural and developmental variations of stomata occurring in the same species, none seem to have studied them for the entire stomatiferous area of the plants investigated. Importance of such an information needs no emphasis for, apart from giving an idea of the behaviour of a given plant part relating to its stomatal variations, it will enable to formulate criteria for utilising even the variable stomata for taxonomic purpose. Tognini, who

was the first to describe organographic stomatal variations, based his conclusions from a study of comparatively larger number of plant parts (cotyledons, leaf, stipule, calyx, corolla and fruit; See in Gupta *et al.*<sup>2</sup>) than others;<sup>2-4,6</sup> even so he left from consideration quite a few parts as the hypocotyl, stem, bracts, peduncle, pedicel, floral disc, stamens, carpels and seedcoat all of which also usually possess stomata. The purpose of this paper is to bring to the fore the full extent of structural