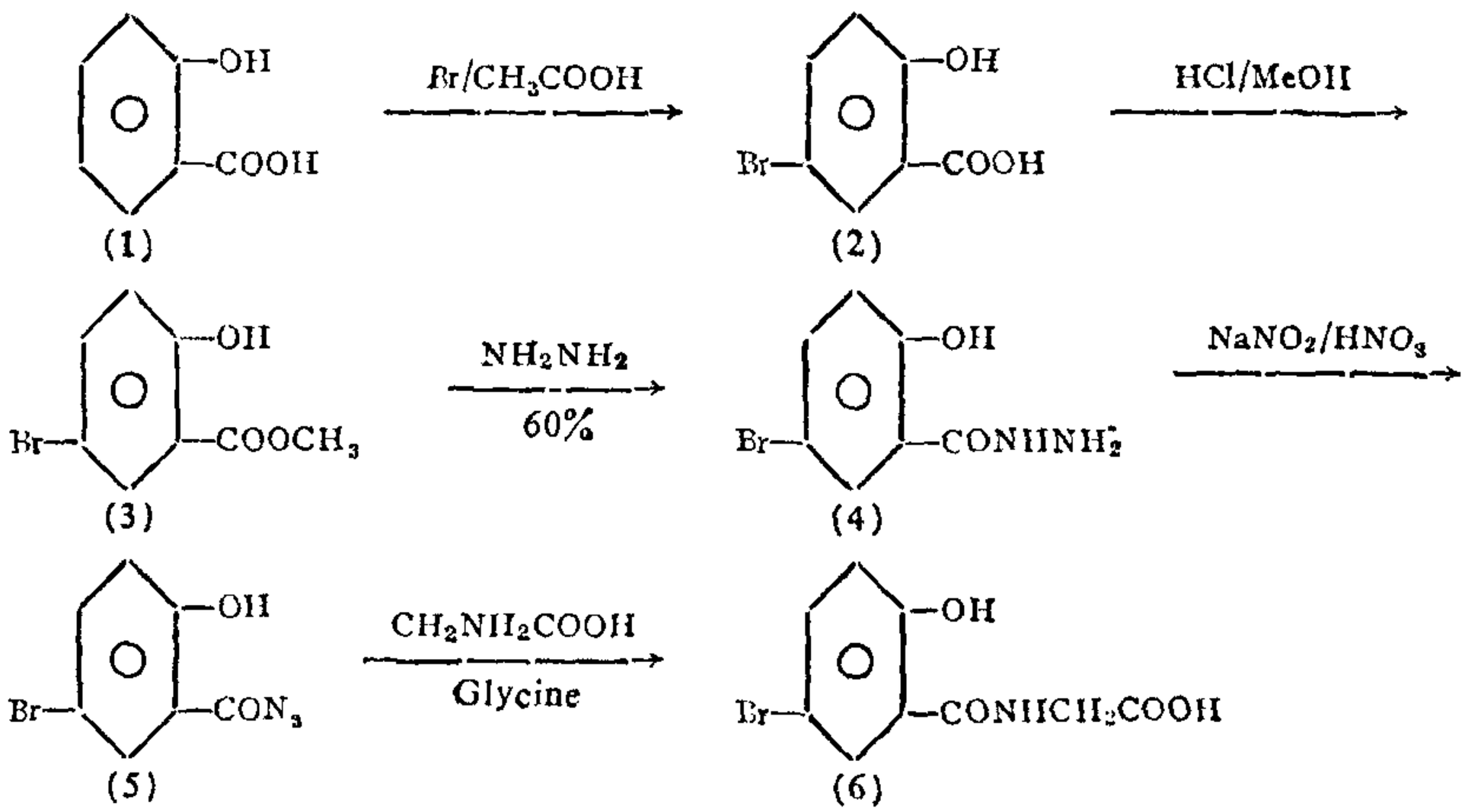
LETTERS TO THE EDITOR

5-BROMOSALICYL AZIDE—A PROSPECTIVE REAGENT FOR IDENTIFICATION OF AMINO-ACIDS

SEVERAL salicyl azides[1,3] have been described as prospective reagents for identification and characterisation of amino-acids. In the salicyl azide[1] and 5-chlorosalicyl azide[2], the azide group reacts with amino group of amino-acid and forms a peptide linkage. In continuation of this work 5-bromosalicyl azide, has now been synthesised and its reactivity with several amino-acids has been studied. These derivatives can be separated from one another by thin layer chromatography.

Salicylic acid (1) was brominated by the method of J. T. Hewitt, J. Kenner and H. Silk[4], when 5-bromo salicylic acid (2) was produced. Methyl 5-bromosalicylate (3) can be obtained from esterification of 5-bromosalicylic acid by Fischer–Speier method. 5-bromosalicyl hydrazide (4) was readily obtained by the action of 60% hydrazine hydrate on methyl 5-bromosalicylate. The hydrazide was converted to 5-bromosalicyl azide (5) by slightly modifying the method employed by S. Bondi[5]. It coupled immediately with amino-acids and gave crystalline derivatives and was employed as a reagent for characterisation of amino-acids.



Procedure

For preparing the derivatives with amino-acids the above moist azide was added in small portions with cooling to a solution of amino-acid (0·2 gm) in normal caustic soda solution (5 ml) and water (10 ml). The mixture was well shaken during addition and was then allowed to stand in ice-bath for two hours and filtered. The clear filtrate was extracted twice with ether to remove unchanged azide. It was then acidified with sulphuric acid (1 ml) and was immediately extracted with ethyl acetate The ethyl acetate layer was then dried over anhydrous sodium sulphate and the excess solvent was removed by distillation. The solid thus separated, was crystallised from alcohol-benzene (1 : 9) mixture.

The following derivatives (Table I) were obtained with the amino-acids and bromosalicyl azide.

All the melting points are un-corrected. The analytical data indicated that the azides react with mono amino-acid as well as diamino-acids. In the case of lysine both the amino groups present react with two molecules of the azide. On the other hand in the case of glutamine and asparagine the azide molecule reacts with only one group though two amino groups are present in their molecules.

The reason is that in the case of glutamine and asparagine an amide group is present. In the amide group the lone pair of electrons of the nitrogen are involved in mesomerism; hence, they are not available for reaction due to direct conjugation with the carbonyl group. So this amino group does not react with the azides. In the case of the amino group attached to –CH– group in lysine, there is no such conjugation and hence its lone pair of electrons reacts with azides.

TABLE I

Sl. No.	Amino acid used	Molecular formula of the derivative with the azide	m.p. °C	R_f Value mm
1.	Glycine	$C_9H_8NO_4Br$	198	0·69
2.	Glutamic acid	$C_{12}H_{12}O_6NBr$	180	0·95
3.	Asparagine	$C_{11}H_{11}O_5N_2Br$	169	0·72
4.	Lysine	$C_{20}H_{21}O_6N_2Br_2$	169	0·58
5.	Glutamine	$C_{12}H_{13}O_5N_2Br$	165	0·92
6.	Leucine	$C_{13}H_{16}NO_4Br$	158	0·97
7.	Isoleucine	$C_{13}H_{15}NO_4Br$	158	0·84
8.	Methionine	$C_{12}H_{14}NO_4SBr$	150	0·76
9.	Cysteine	$C_{10}H_{10}NO_4SBr$	138	0·89
10.	Valine	$C_{12}H_{14}NO_4Br$	135	0·71
11.	Alanine	$C_{10}H_{10}NO_4Br$	115	0·76

The present reagent is advantageous as compared to other reagents mentioned in the Literature.

The various amino-acid used in this work were gifted by the Late Dr. Ajai Haksar of the Worecster foundation for experimental Biology, Shrewsbury (U.S.A.). Micro-analyses were kindly carried out at C.D.R.I., Lucknow. The award of a U.G.C. research scholarship and a research grant to one of us (S. S.) is also gratefully acknowledged.

School of Studies in Chemistry, Jiwaji University, Gwalior, (MISS) S. SAXENA.
C. N. HAKSAR.
April 19, 1978.

1. Saxena, S. and Haksar, C. N., *J Jiwaji University*, 1976 (I), 4, 36.
2. — and —, *Ibid.*, 1976 (II), 4, 45.
3. — and —, *J. Indian Chem. Soc.*, 1978, **55**, 300.
4. Hewitt, J. T., Kenner, J. and Silk, H., *J. Chem. Soc.*, 1904, p. 1228.
5. Bondi, S., *Z. Physiol. Chem.*, 1907, **52**, 172.

A NOVEL ARRANGEMENT OF VASCULAR TISSUE IN SOME ORCHIDS

THE authors in their studies on the root tubers of some orchids of medicinal importance, *viz.*, *Habenaria* genus encountered a characteristic novel arrangement of vascular tissue hitherto unreported in the literature.

The thick distal regions of the tubers of *H. edgeworthii* Hook. f. and *H. marginata* Colebr. showed a typical condition with 8–19 steles in former (Fig. 1) and 6–9 in the latter, arranged in a ring and each stele

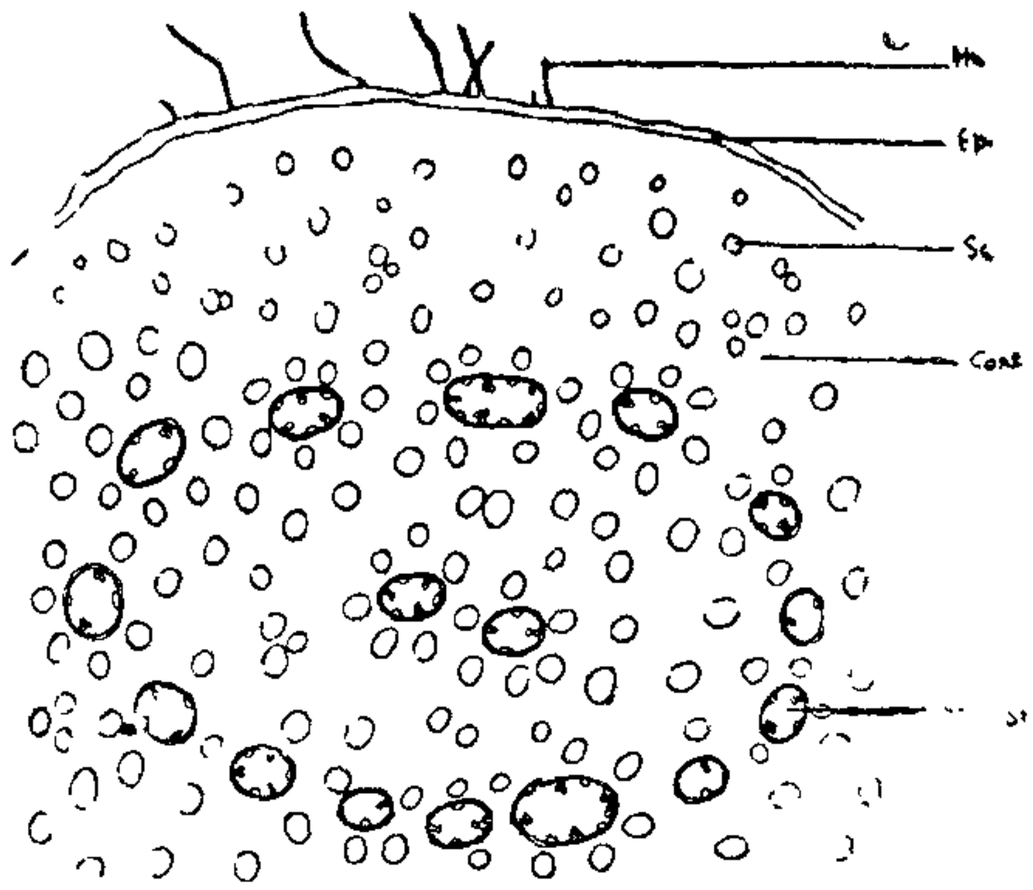


FIG. 1. A t.s. through distal portion of root tuber (diagrammatic), × 25. (*Epi.*, Epiblema; *Cort.*, Cortex; *Hr.*, Hair; *Sc.*, Secretion canal; *Ste.*, Stele).

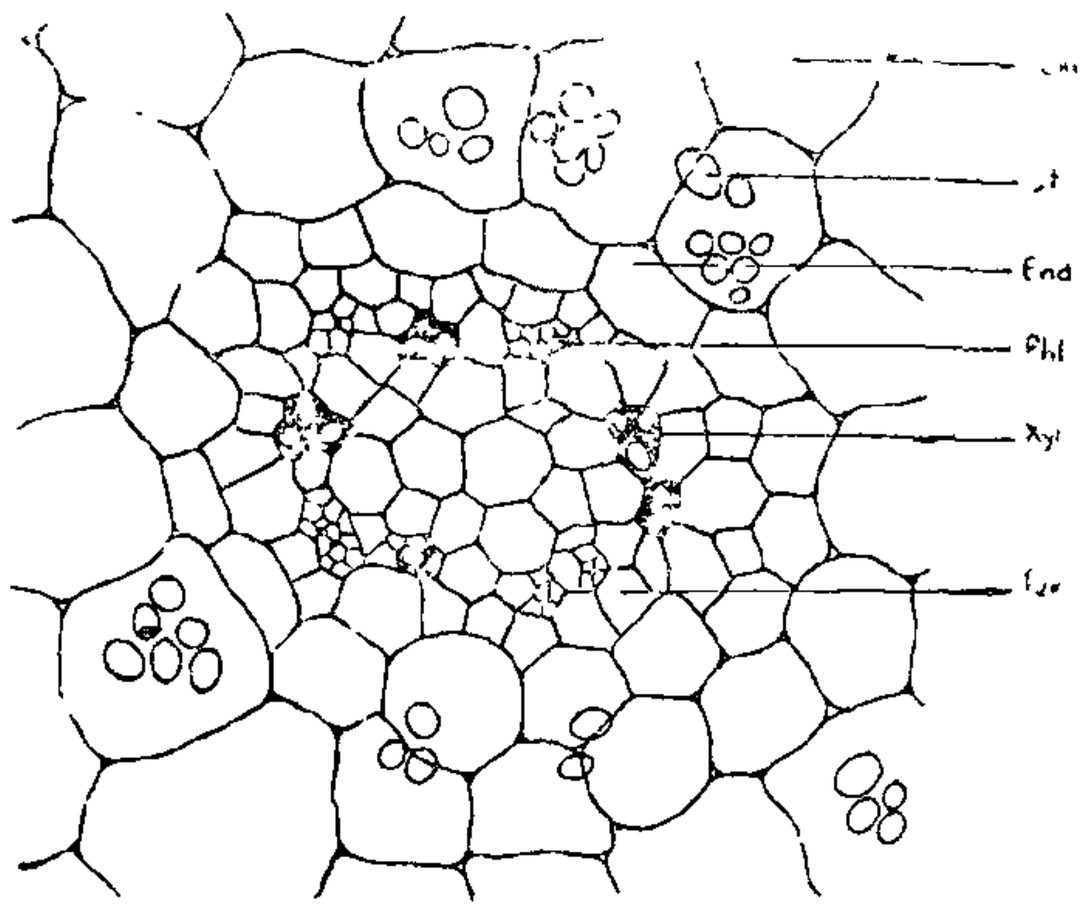


FIG. 2. Details of a portion of Fig. 1 showing tetrarch condition, × 270. (*Cort.*, Cortex; *End.*, Endodermis; *Per.*, Peridycle; *Phl.*, Phloem; *St.*, Starch grain; *Xyl.*, Xylem).

in itself presents a clear mono- to pentarch condition (Fig. 2). Further in addition to the steles arranged in a ring, 1–8 steles each with mono- to triarch condition in *H. edgeworthii* and a single stele with mono- to diarch condition in *H. marginata* are found distributed within the parenchyma in the central region of the tuber. However, in the slender proximal region a normal stelar structure common to monocots was observed.

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi-5, *June* 27, 1978.
V. K. LAL.
A. K. WAHI.
R. L. KHOSA.