FIG. 1.

Circular paper chromatogramof a mixture of Alanine, Glycine, Leucine and Valine.

A-alanine; G-glycine; L-leucine; V-valine.

R_f Values

Alanine	..	0·44
Arginine (1)	..	0·32
Asparagine	..	0·31
Aspartic acid	..	0·37
Glutamic acid	..	0·44
Glycine	..	0·37
Histidine	..	0·50
Leucine	..	0·73
Iso-leucine	..	0·70
Nor-leucine	..	0·75
Lysine	..	0·45
Methionine	..	0·92
Ornithine (2)	..	0·25
Phenylalanine	..	0·70—0·75
Proline	..	0·49
Serine	..	0·40
Threonine	..	0·44
Tryptophane	..	0·70
Tyrosine	..	0·58
Valine	..	0·62

(1) Applied as monohydrochloride

(2) Applied as hydrobromide

values obtained for the several amino acids using butanol-acetic acid-water as solvent.

In general the values are found to vary slightly from those reported by other workers by descending and ascending paper chromatographic techniques.

Amino acids which are not separated into distinct zones can be resolved by multiple development technique.[4] Distinct improvement in the separation of the amino acids is seen after each development. Fig. 2 illustrates the application

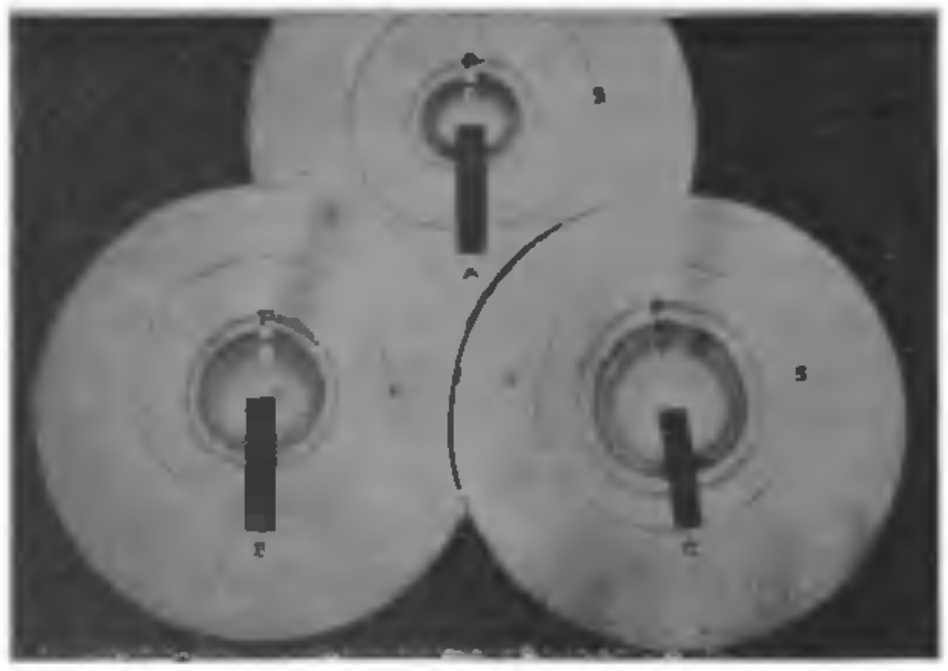

FIG. 2

Multiple development of the chromatogram of a mixture of Proline, Arginine and Ornithine.

A. First development

B. Second development

C. Third development

P. Proline

A. Arginine

O. Ornithine

S. Solvent boundary

of this technique to the separation of arginine and ornithine, which are not separated into two distinct zones by first development. By repeating the development, however, the two circular zones relating to the amino acids are separated from each other, after the third development.

Thus, amino acids can be separated by means of this technique and it is of particular interest that several chromatograms can be carried out at the same time and in short period. It is capable of wide application to the amino acid analysis of biological fluids.

Full details of the method will be published elsewhere.

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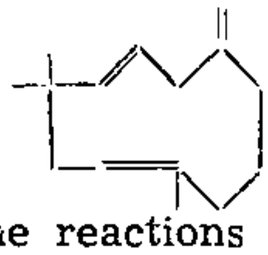
Ind. Institute of Science,
Bangalore,
October 4, 1951.

1. Rutter, L., *Nature*, 1948, **161**, 435; *Analyst*, 1950, **75**, 37. 2. Rao, P. S. and Beri, R. M., *Proc. Ind. Acad. Sci.*, 1951, **33**, 368. 3. Tonnies, G. and Kolb, J. J., *Anal. Chem.*, 1951, **23**, 823. 4. Jeanes, A., Wise, C. S., and Dimler, R. J., *Ibid.*, 1951, **23**, 415.

STRUCTURE OF HUMULENE

HUMULENE on treatment with Aschan's reagent yields a crystalline, tricyclic, fully saturated alcohol, m.p. 116° which appears to be identical with the α-caryophyllene alcohol of Asahina and Tsukamoto.[1] This reaction is of importance, since it transforms a monocyclic hydrocarbon into a tricyclic derivative. If the molecule of

humulene be represented by a system with more than 10 carbon atoms in a cycle, it is not difficult to visualise such a transformation which otherwise, normally is uncommon. Taking into account its close occurrence with β-caryophyllene, and the structure of the latter hydrocarbon as proposed by Sorm, Dolejs and Pliva[2] and modified by Dawson, Ramage and Wilson,[3] the formula (I) for humulene appears to be attractive.



(I)

This explains the reactions of the hydrocarbon known so far. The formation of lævulinic aldehyde[4] can be explained if the exo-cyclic double bond becomes endocyclic. If such a system can be transformed into a *bicyclo*-compound, we should normally expect a potential naphthalene or a potential azulene derivative. Humulene on treatment with *p*-toluene sulphonic acid gives a *bicyclo*-humulene which, on dehydrogenation over Pd-C at 325-335°, gives an azulene.

Though the present studies are far from complete, it has been thought desirable to place the results of the investigation on record in view of the publication of an advanced communication by Clemo and Harris[5] on this subject.

Controlled oxidation of a dihydrohumulene and further work is in progress to throw more light on the subject.

The humulene for this investigation was isolated from the essential oil of Wild Ginger[6] (*Zingiberzerumbet Smith*) and had b.p. 104°/3 mm., n^{25}, 1·5005; d_{25}^{25} 0·8900; and $[\alpha]_D^{25}$ −0·9°.

Full details will be published elsewhere.

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1. *J. Pharm. Soc.*, Japan, 1922, No. 484, 463. 2. *Coll, Czech. Chem. Comm.*, 1950, **15**, 186. 3. *Chem and Ind.* **1951, 464.** 4. Clemo and Harris, *J.C.S.*, 1951 22. 5. *Chem. and Ind.*, 1951, 799. 6. *cf.* Paribar and Dutt *Indian Soap Journal*, 1950, **16**, 123.

ANTITUBERCULAR ACTIVITY OF *CUCURBITA PEPO*

THE following communication deals with the anti-tubercular activity of *Cucurbita pepo*, a vegetable widely recommended, for arresting haemoptysis and controlling the disease process in pulmonary tuberculosis.[1,2]

C. pepo. Roxb. 700. *N. O. Cucurbitacæ* (Syn. white melon, *Budi-gumbala, Kushmanda*) is cultivated in gardens throughout India. The fruit is used as a household vegetable. Extract from the ripe fruit is regarded as a diuretic, tonic and is used in painful micturition, calcareous affections and general urinary disorders, besides its specific use in tuberculosis.

The outer skin and the inner seeds having been removed, the fleshy part of a ripe, well-preserved fruit was minced with an equal quantity of water in a waring blender; the extract was concentrated to half its volume over a water-bath and strained through muslin. Further concentration was carried out under reduced pressure and finally dried over $CaCl_2$ in a desiccator. The yield was 10% of the original weight. A brown sweet-smelling syrupy liquid of the consistency of treacle, of pH 6·8, was the product obtained.

"In vitro" tuberculostatic activity.—This was first determined in Youman's synthetic liquid media using D_{13} and H_{37} R_v strains of *Myco. tuberculosis*, by methods already described.[3] The extract inhibits the growth of these virulent strains completely in a 1/10,000 dilution and retards more than 50% of the growth in a 1/100,000 dilution.

The tuberculostatic action was next tested by incorporating the various dilutions of the extracts in a rich nutrient solid media (Petrick's[4] media gave the best results in our studies), and seeding varying amounts of different strains of Myco tuberculosis. Tests were made in duplicate, the results being noted at the end of 3 weeks. Table I summarises the results obtained against an inocula of 0·1 mg. of tubercle bacilli.

TABLE I

Anti-Tubercular Activity of a Watery Extract of C. pepo *in Petrick's media*

Concentration of the extract	Strain of Myco. tuberculosis		
	D_{13}	$H_{37}R_v$	B.C.G.
1/100	−	−	−
1/1,000	−	−	+
1/10,000	+	2+	2+

− No growth; + to 2+ various grades of growth.

The growth of the fresh virulent strain D_{13} was partially inhibited at 1/10,000 dilution. Complete inhibition of both the virulent strains was obtained in 1/1,000 dilution while the action against the non-virulent B.C.G. was of a much lower order.

The general anti-bacterial activity of the extract against some non-acid-fast organisms was