TABLE I
Composition of solvent mixtures

Solvent mixtures	1	2	3	4	5
Butanol ..	10	10	10	10	5
Gasoline ..	..	..	..	..	5
Acetic acid ..	2·5	1·2	..	..	..
Formic acid ..	..	..	2	2	2
Water ..	10	4	6	5	5

Lugg and Overell[2] showed that 1-2 moles of acetic acid per litre of the phase, gave the best separation. By trying various concentrations of acetic acid, we found that mixture No. 2 (Table I) gave a better separation than mixture No. 1. Four volumes of water were taken for mixture No. 2, since this amount of water was found to be just sufficient to give two separate layers when mixed with 10 parts of butanol and 1·2 parts of acetic acid.

Similarly in the formic acid series the mixture composed of 10 butanol : 2 formic acid : 5 water, gives the best separation and also the yellow bands at the bottom of the chromatogram totally disappear. Besides, the substitution of formic in place of acetic acid gives better and more well-defined spots and Rf values (Table II) which are reasonably wide apart. A trial run with a solvent mixture of gasoline and butanol[3] did not yield any encouraging results in our hands (Table II). Best resolutions were obtained with solvent mixture No. 4; we have accordingly adopted this solvent mixture for all our studies.

Experimental.—The fruits chosen for our investigation were :

(1) *Averrhoa carambola* Linn.; (2) *Citrus decumana* Linn. (Pomello); (3) *Citrus limonum* (Lemon); (4) *Citrus medica* Linn. (Bitter lime); (5) *Citrus sinensis* (Orange); (6) *Morus indica* Linn. (Mulberry leaf); (7) *Phyllanthus emblica* Linn. (Gooseberry); (8) *Phyllanthus simplex* Retz.; (9) *Punica granatum* Linn. (Pomegranate); (10) *Tamarindus indica* Linn. (Tamarind); (11) *Vitis vinifera* Linn. (Unripe grapes); (12) *Zizyphus jujuba* Lamk.

Fleshy portions of the fruits (10-15 g.) were ground up with 50 per cent. alcohol acidified with acetic acid, and the mash strained through cheese cloth. The extract was made up to 20 ml., centrifuged and the clear supernatant used for papyrographic studies (preserved in a refrigerator in small bottles). Measured quantities of the extracts were spotted on rectangular sheets (20 × 22 cm.) of Whatman No. 1,

PAPYROGRAPHIC CHARACTERISATION AND ESTIMATION OF ORGANIC ACIDS IN PLANTS

LUGG AND OVERELL[1] modified the composition and the nature of the solvent phase for the papyrographic detection and separation of non-volatile organic acids by substituting formic acid in place of acetic acid, on the basis of the fact that formic acid had a higher ionization constant ($K = 2 \cdot 0 \times 10^{-4}$) than acetic acid ($K = 1 \cdot 8 \times 10^{-5}$); they reported that the former is effective in suppressing the comet effect. During our studies on the organic acid make up of plant tissues, we were confronted with the problem of determining the most effective composition of the solvent mixture for securing discrete separations and compact and well-defined spots. The following solvent mixtures were compounded and employed for papyrographic separation of organic acids,

TABLE II

** Rf values with different solvent mixtures*

Organic acids	Solvent mixture No. 1	Solvent mixture No. 2	Solvent mixture No. 3	Solvent mixture No. 4	Solvent mixture No. 5
Aconitic ..	0·64	0·63	..	0·78	0·61
Citric ..	0·39	0·26	..	0·37	0·20
Fumaric ...	0·72	0·73	..	0·86	0·76
Glutaric ..	0·79	0·80	..	0·80	0·70
Itaconic ..	0·79	0·77	..	0·81	0·70
Lactic ..	..	0·63	..	0·77	0·60
Maleic ..	..	0·32	..	0·46	0·34
Malic ..	..	0·37	..	0·44	0·27
Malonic ..	..	0·42	..	0·60	0·58
Oxalic ..	..	0·17	..	0·05	..
Succinic ..	..	0·73	..	0·72	0·68
Tartaric ..	.	0·22	..	0·23	0·22
Tricarballylic ..	..	0·63	..	0·67	0·61
Remarks	Tailing; spots not clear; a yellow band at the bottom	Tailing; the yellow band present	Unsatisfactory; spots are not well-defined; tailing	No tailing; spots compact and clear; yellow band at the bottom absent	..

* Average for four values

rolled into the form of cylinders. After equilibration with the vapour phase in the developing chamber, papyrograms were developed with the solvent mixture No. 4 at room temperature (25-26° C.) which generally took 5-6 hours. The papyrograms were then air-dried over night and later oven-dried at 80° C. for 30 minutes. After spraying with bromo-cresol-green (40 mg./100 ml. 95% alcohol).[4,5] The presence of the different acids was indicated by well-defined lemon yellow spots against a blue background, and by developing papyrograms of known reference mixtures along with those of extracts and by a reference to the Rf values determined by us (Table II), the acids occurring in the mixture could be characterized. The area occupied by a given spot was found to give us a measure of the quantity of the acid present in the extract. Since the quantity of the extract used for spotting is known, it has been possible to express the quantity of the constituents of the mixture as milligrams per ml. The results are given in Table III.

TABLE III

Plant material	Organic acids mg./ml.				
	Citric	Malic	Oxalic	Succinic	Tartaric
1 *Averrhoa carambola* Linn.	..	5·39	..	..	..
2 *Citrus decumana* Linn.	..	..	2·50	..	..
3 *Citrus limonum*	74·36	4·01	20·00	7·50	..
4 *Citrus medica* Linn.	18·12	1·37	2·18	..	..
5 *Citrus sinensis*	3·67	1·07	..	..	..
6 *Morus indica* Linn.	..	..	..	0·52	0·36
7 *Phyllanthus emblica* Linn.	9·60	13·50	3·53	..	1·29
8 *Phyllanthus simplex* Retz.	..	..	15·00	..	..
9 *Punica granatum* Linn. (acid variety)	37·57	..	1·2	..	..
10 *Tamarindaus indica* Linn.	..	1·37	..	..	10·63
11 *Vitis vinifera* Linn.	..	5·09	..	..	4·88
12 *Zizyphus jujuba* Lamk.	12·17	3·46	4·47	..	..

Of the citrus fruits examined, the *Citrus limonium* contains the highest amount of citric and also a surprisingly high proportion of oxalic acid. Among the non-citrus fruits, *Punica granatum*, the acid variety of pomegranate, contains citric acid to the extent of 95 per cent. with a trace of oxalic acid. It is surprising that *C. decumana* does not contain any citric but only oxalic acid. Tamarind contains, as expected, about 88 per cent. of tartaric acid, malic accounting for the remaining portion of the acidity. No other acids are present. The grape contains exclusively the tartaric and malic acids in almost equal proportions. The two members of the phyllanthus family—*P. emblica* and *P. simplex*—show interesting differences. The medicinally important *Emblica* show high proportions of the citric and malic

acids with low percentages of oxalic and tartaric acids while simplex consists of only oxalic acid to the absolute exclusion of other acids.

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