RELATIONSHIP OF THE ORGANIC ACIDS OF TOBACCO TO THE INORGANIC BASIC CONSTITUENTS[1]

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Introduction

There is little doubt that one of the important functions of the organic acids commonly found in relatively substantial proportions in leaf tissues is to provide a means whereby the hydrogen-ion activity of the solution in the cells is maintained within the proper limits for the particular species. There is considerable literature on the buffer capacity of plant juices (**3, 4, 5**), and it is obvious that the combined effects of the partially neutralized organic acids, and of the phosphoric acid, as well as of the amino acids, and posssibly, to some extent, of the soluble proteins result in systems that are remarkably stable with respect to change in hydrogen-ion activity.

Owing to the inadequacies of the methods available until recently, few reliable studies have been made of the detailed organic acid composition of leaves, and for determinations of total acidity many investigators have depended entirely upon titration of water extracts of the tissues. Such data furnish a measure merely of that part of the water-soluble acids that remains unneutralized at the reaction of the cells and give little indication of the total quantity present either as soluble or insoluble salts. In order to ascertain the true organic acidity it is necessary to liberate the acids from combination and isolate them as a group; the total quantity present can then be determined by titration between properly selected pH limits. With this information available it becomes possible to investigate the relationships between the chief acid- and base-binding substances of the tissue.

Data obtained in the course of a study of these factors in tobacco leaf tissue are described in the present communication. We were fortunate in having at our disposal a series of samples derived from plants that had been grown with especially careful control of the inorganic constituents of the fertilizer applied. Complete analyses of the ash of these samples had been made by Dr. E. M. BAILEY of the Connecticut Agricultural Experiment Station and published in connection with BAILEY and ANDERSON's studies (**1**) of the effect of various soil treatments on the technical quality of the leaf. We are indebted to them for permission to carry out organic acid analyses of these samples and to recalculate and quote their data.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

Experimentation

The samples used in the experiments consisted of leaves that had been subjected to the processes of curing and of fermentation. It was therefore desirable first to establish the nature of the effect of these operations on the organic acids. Samples of tobacco leaves picked the same day were allowed to cure for various periods in the customary way. Each sample consisted of 60 leaves of approximately the same total initial weight. The results of the analyses were calculated upon an arbitrary basis of 1 kilo of original fresh weight of each individual sample. The data referring to the nitrogenous constituents and the carbohydrates have been discussed in a previous publication (**7**). The results of the determinations of the total organic acidity are given in table I.

TABLE I

TOTAL ORGANIC ACIDITY OF TOBACCO LEAVES AT VARIOUS STAGES IN THE CURING PROCESS

HOURS	ACIDITY*
	meq./kgm.
0	320
41	367
64	340
87	285
111	320
159	272
185	275
207	347
231	357
279	308
303	339
Average	320.9 ± 22.1

* Data are expressed in milliequivalents per kilogram of original fresh weight of each sample.

These figures show that little or no change occurs in the total organic acidity throughout the period of curing. The probable error is ± 6.9 per cent., which is the order of precision to be expected for measurements of this type. Alterations in the relative proportions of individual acids may, and in fact do, occur, but there is no substantial conversion of organic acids into nonacidic metabolic products, nor is there evidence for the accumulation of acid end products of the metabolism of other substances.

The information available on the effect of fermentation on the organic acids is very limited. HALEY, NASSET, and OLSON (**2**) carried out determinations of the ether-soluble organic acidity of parallel samples of cured and fermented tobacco, employing a method not unlike that used in this laboratory. The average of nine determinations made on web tissue before and after fermentation differed by only 3 per cent., and it seems fair to assume therefore that such changes as may occur during fermentation are of a minor nature. This conclusion is supported by inferences drawn from the reaction of the tissues. The reaction of water extracts of our fermented samples ranged from pH 6 to 6.5—values of exactly the same order of magnitude as those of extracts from many samples of cured tissue. Important alterations in the proportion of organic acids present would have changed this reaction appreciably inasmuch as the inorganic basic constituents did not change. For the present purposes, however, comparisons are to be made between samples, all of which had been subjected to fermentation in the same way; minor changes that may have taken place in the organic acids accordingly have no significance.

The samples were derived from two different crops, 1924 and 1927, respectively a dry and a rainy season, and represented a considerable variety of cultural treatments. The two crops differed widely in the technical quality of "fire-holding capacity," the 1927 crop being classified as good, the 1924 crop as poor in this relation. The soil treatments of each individual plot were the same in each year, and had been maintained the same for the crops grown during the intervening years on the same plots. All samples were graded by experts, and the so-called darks, "D," and seconds, "S" were employed for the present work. These two grades represent leaves derived respectively from the upper and from the lower parts of the plant.

The detailed analyses of the ash and of the intact tissue were recalculated for the present purpose in terms of milliequivalents per 100 gm. of dry leaf. The positive ions calcium, magnesium, potassium, nicotine, and ammonium, and the negative ions phosphate, chloride, sulphate, and nitrate, were employed. Phosphorus and nicotine were reckoned as monovalent ions in the calculations because the tissue reactions were close to pH 6. The analytical figures for sulphate reported by Bailey and Anderson represent both organic and inorganic sulphur, but a study of the sulphur distribution of the samples showed that approximately 70 per cent. was in the form of inorganic sulphate; much of the balance doubtless represents sulphur combined in proteins. The factor 0.7 was therefore used to correct the total sulphur determinations of Bailey and Anderson in order to arrive at an approximation to the quantity of inorganic sulphate. An analogous correction was not applied to the phosphate data although the determinations represent total phosphorus in the ash, and a part of this may have originated from organic phosphorus compounds. It is assumed that all of the phosphorus represents monovalent phosphoric acid radicals as there is little doubt from the nature of the known organic phosphorus compounds of plant tissues that a part of the phosphorus even in such compounds fulfills this function. In any case, the error involved in this assumption has little effect upon the conclusions to be drawn as phosphoric acid forms so small a part of the total inorganic acidity present. The nature of the compounds of silicon in the tissues presents a very puzzling problem, and it would appear from the present data that very little if any of the silicon dioxide determined in the ash represents silicic acid or analogous acidic substances.

The sum of the positive ions present in the ash, together with the ammonia and nicotine, may be taken to represent the total acid-binding capacity of the tissues. The sum of the inorganic negative ions represents the base-binding capacity of the tissues due to inorganic acids. In all cases there is a large excess of positive ions, and it is the purpose of the present paper to show that this excess is closely related to the quantity of ether-soluble organic acids in the intact tissue.

It should, perhaps, be emphasized that the comparison is made between the excess of positive ions present at approximately the tissue reaction of pH 6 and the *whole* of the ether-soluble organic acids that can be titrated between the limits pH 7.8 and 2.6. This involves the assumption that the proportion of organic acids in combination with bases is substantially fixed, notwithstanding possible variation from sample to sample in the chemical composition of this group of substances. Furthermore, in the calculation of the bases it is assumed that the inorganic constituents of the ash are all present in the intact tissue in ionic form capable of salt formation. This is certainly not entirely true; for example, a part of the magnesium is involved in the fresh tissues in complex combination in the form of chlorophyll. The fate of this part of the magnesium after the technical processes of curing and fermentation have been carried out is entirely unknown.

The data assembled in table II permit a comparison between the excess acid-binding capacity of the individual samples from the 1927 crop and the total organic acidity as determined by the method of PUCHER, VICKERY, and WAKEMAN (**6**) after ether extraction.

The degree with which one variable depends on the variation of another is probably most easily appreciated from a consideration of the correlation coefficient calculated in the customary way. In the present case we have two sets of data, one obtained from plants fertilized with various potassium compounds, the other with lime. Each set of 12 samples contains 6 lots of leaves graded as "darks" and 6 graded as "seconds." These grades imply a difference in position on the plant as well as intangible elements of technical "quality," but for the present purpose this may be neglected and the two series grouped together.

The correlation coefficient between the excess positive ions and the total organic acidity for the 12 samples from plants grown on the potash plots is 0.833 ± 0.060; that for the other set is 0.905 ± 0.035. A similar calculation for the entire set of data from the 1924 crop (13 samples) gave a correlation coefficient of 0.812 ± 0.067. These coefficients indicate that the quantities are closely dependent on each other.[2]

Although the statistical analysis of the data leads to the conclusion that the two variables are closely interdependent, it in no way suggests which is the independent and which the dependent variable. It is not possible to conclude that certain plants absorbed more basic ions from the soil and therefore elaborated more organic acids. It may be equally true that certain plants manufactured more organic acids and therefore absorbed more inorganic base from the soil. Some entirely different factor may indeed have been the true independent variable, and it is quite possible that the mutually related organic acidity and excess basic ion content varied in response to the changes in this other factor.

Inspection of the data of table II shows that the inorganic acidic components of the tissue (negative ions) play a minor rôle in maintaining the balance between the positive and negative ions of the tissue. The average of the total inorganic anions amounts to only about one-sixth of the average total cations in the samples from the potash plots and to one-tenth for the samples fertilized with lime. Organic acidic substances, particularly in the latter case, obviously dominate the situation.

It must be emphasized that we are in a position to deal from the analytical point of view only with that part of the organic acids of the tissues that can be extracted with ether under suitable conditions. No accurate estimate can at present be made of base-binding substances that cannot be extracted with this solvent. The tobacco leaf contains considerable quantities of pectins and allied compounds, some of which possess base-binding properties. If the assumptions upon which the present calculations have been founded are correct, it would appear that roughly one-third of the excess positive ions are, on the average, combined with acidic substances other than ether-soluble organic acids.

The acids actually determined by the method employed are chiefly malic, oxalic, and citric; these three make up about four-fifths of the ether-soluble acidity. The exact chemical nature of this group is, however, immaterial to the present discussion.

If it be accepted that the high correlation between the excess positive ions and ether-soluble organic acidity in tobacco leaf tissues demonstrates a mutual interdependence between these quantities, inferences may be drawn from average values and extended to the consideration of other sets of

[2] The high significance of these coefficients may be appreciated from comparison with FISHER's table (SNEDECOR, G. W. Statistical methods. Ames, 1937, p. 125). For 10 degrees of freedom the coefficient at the 1 per cent. level of significance is 0.708, for 11 degrees of freedom it is 0.684.

TABLE II

RELATIONSHIP BETWEEN POSITIVE AND NEGATIVE IONS OF ASH, TOGETHER WITH AMMONIA AND NICOTINE, AND TOTAL ORGANIC ACIDITY OF INTACT TISSUE IN SAMPLES OF FERMENTED TOBACCO LEAF FROM CROP OF 1927

POTASH SERIES*						LIME SERIES*					
SAMPLE NO.	GRADE	TOTAL POSITIVE IONS	TOTAL NEGATIVE IONS	EXCESS POSITIVE IONS	TOTAL ORGANIC ACIDITY	SAMPLE NO.	GRADE	TOTAL POSITIVE IONS	TOTAL NEGATIVE IONS	EXCESS POSITIVE IONS	TOTAL ORGANIC ACIDITY
608	D	385.8	81.4	304	175	620	D	430.4	34.9	396	245
610	D	402.0	65.2	337	207	622	D	396.4	65.9	331	223
612	D	385.7	64.3	321	177	624	D	413.1	39.8	373	272
614	D	403.7	63.7	340	176	626	D	388.3	44.4	344	216
616	D	430.4	84.9	346	205	628	D	450.8	27.7	423	304
618	D	407.4	70.8	337	206	630	D	388.3	40.1	348	212
Average		402.6	71.7	331	191			411.2	42.1	369	245
609	S	443.0	98.0	345	183	621	S	470.2	33.3	437	302
611	S	445.7	87.7	358	250	623	S	411.1	50.5	361	250
613	S	424.6	73.6	351	219	625	S	444.8	29.0	416	290
615	S	418.1	57.9	360	213	627	S	383.7	47.6	336	253
617	S	434.6	69.6	365	262	629	S	488.3	27.0	461	335
619	S	435.7	53.3	382	211	631	S	404.1	23.7	380	272
Average		433.6	73.3	360	223			433.7	35.2	398	284
	Correlation coefficient r = 0.833 ± 0.060						Correlation coefficient r = 0.905 ± 0.035				

* Figures are milliequivalents per 100 gm. of dry tissue.

samples. It may be useful to present the data from this point of view. In table III are shown the average values of the organic acidity and of the excess positive ions of samples of grade D from two crops fertilized each in two ways. In the upper portion of the table are details from which the relative order of magnitude of the individual bases and of the inorganic acidity may be appreciated. Calcium and potassium clearly occupy the dominant position among the bases.

The organic acidity of the samples grown on the potash plots in 1924 was distinctly higher than that of the samples grown in 1927. In the later crop, there was a marked decrease in total positive ions, partly due to a diminution in calcium content, and there was a corresponding decrease in inorganic acidic ions. The compensation was not, however, complete so that the ratio between the excess positive ions and the organic acidity changed from 1.52 to 1.73.

The samples from the lime-fertilized plots showed a slight increase in organic acidity and in excess positive ions. There was a marked decrease in calcium and potassium in the 1927 crop which was only partially compensated by an increase in magnesium. The substantial drop in inorganic negative ions, however, completed the compensation and preserved the balance between the excess positive ions and the organic acids. The ratio did not change significantly.

If the potash series of 1924 is compared with the lime series of the same

TABLE III

RELATIONSHIP BETWEEN POSITIVE AND NEGATIVE IONS OF SAMPLES OF TOBACCO OF GRADE D DERIVED FROM THE CROPS OF 1924 AND 1927

	POTASH SERIES*		LIME SERIES*	
	1924	1927	1924	1927
Calcium	180	148	200	161
Potassium	166	173	150	128
Magnesium	67.7	55.6	54.7	90
Nicotine	14.6	10.6	16.3	13.7
Ammonia	33.2	15.6	39.3	18.6
Total positive ions	462	403	460	411
Total negative ions (inorganic acidity)	107	71.7	110	42
Excess positive ions	355	331	350	369
Organic acidity	234	191	236	245
Ratio $\frac{\text{excess positive ions}}{\text{organic acidity}}$	1.52	1.73	1.48	1.51

* Figures are averages of from four to six individual samples and are expressed in milliequivalents per 100 gm. of dry tissue.

year, it is clear that there was no significant change in the organic acidity nor in the excess positive ions. The inorganic acidity is also practically the same, the slight difference in excess positive ions being attributable to minor differences in calcium and potassium. But if the potash series of 1927 is compared with the lime series of that year, there is an important increase in organic acidity in the samples from the lime plots. There is only a small increase in total positive ions, but there is a substantial diminution in the inorganic negative ions, so that the excess of positive ions increases materially. Nevertheless, the change falls a little short of being strictly proportional to the change in organic acids, the ratios being 1.73 and 1.51 respectively.

A similar comparison between the "D" samples and the "S" samples from the 1927 crop is shown in table IV. These grades to a considerable extent represent position of the leaves on the plant, the "S" grade being mainly bottom leaves, the "D" grade, upper leaves. The bottom leaves from the potash plots contain more organic acids than top leaves from plants fertilized in the same way. The inorganic negative ions are practically the same, and it is clear that the balance of ions is maintained by the organic acids. The same is true of the two sets of samples from the lime plots. Differences between the lime and potash samples within each group are mostly caused by the lower inorganic negative ions in the lime series with correspondingly higher organic acids.

TABLE IV

RELATIONSHIP BETWEEN POSITIVE AND NEGATIVE IONS OF SAMPLES OF GRADE D AND GRADE S FROM THE CROP OF 1927

	"D" samples* Top leaves		"S" samples* Bottom leaves	
	Potash series	Lime series	Potash series	Lime series
Calcium	148	161	177	180
Potassium	173	128	181	120
Magnesium	55.6	90	62.7	112
Nicotine	10.6	13.7	5.7	9.9
Ammonia	15.6	18.6	7.4	11.5
Total positive ions	403	411	434	433
Total negative ions (inorganic acidity)	71.7	42	73.3	35.2
Excess positive ions	331	369	360	398
Organic acidity	191	245	223	284
Ratio $\frac{\text{excess positive ions}}{\text{organic acidity}}$	1.73	1.51	1.61	1.40

* Figures are averages of six samples and are expressed in milliequivalents per 100 gm. of dry tissue.

It is apparent that the ether-soluble organic acidity of tobacco leaf tissue may vary between relatively wide limits, and that this variation is associated with the manner in which the plants have been fertilized. The data indicate with some clearness that the fluctuations are in the direction which, from the chemical composition of the tissue, may be expected to maintain an equilibrium between the positive and negative ions such that the hydrogen-ion activity of the tissues does not vary in any significant manner. Many factors undoubtedly come into play, but the nature of the inorganic ions that are assimilated and the total amount present have a direct influence. The organic acids are therefore definitely concerned in the problem of inorganic nutrition, and a comprehensive study of the manner in which these substances may vary in response to the cultural treatment of the plant is much to be desired.

Summary

1. If it be assumed that the major constituents of the ash of cured and fermented tobacco leaves, with the exception of silicon dioxide, represent respectively basic or acidic ions combined essentially as salts in the cells, and if, in addition, allowance is made for the nicotine, ammonium, and nitric acid of the tissues, it is possible to calculate the total positive ions and the total negative ions present in terms of chemical equivalents.

2. In all cases examined there is a large excess of positive ions and the quantity so found is closely correlated with the quantity of ether-soluble organic acids as determined by a suitable titration method.

3. The organic acids occupy a dominating position with respect to the balance of positive and negative ions in the tissues, and it is inferred that these substances are closely concerned in the phenomena of inorganic nutrition.

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