PERMEABILITY—A PRIME FACTOR IN EXTRACTION OF CHLOROPLAST PIGMENTS

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The extraction of chloroplast pigment from dried nettle leaves is accomplished best in 80–90% aqueous acetone, in 90% aqueous ethanol and in absolute methanol (Willstätter and Stoll, 1913). Just why 80–90% aqueous acetone is better than anhydrous acetone, why 90% aqueous ethanol is better than absolute ethanol, and why absolute methanol is better than any of its aqueous dilutions has never been satisfactorily explained. Willstätter and Stoll (1913, p. 57) explained it on the basis that "water added to the organic solvents dissolves mineral salts, as for example, potassium nitrate, from the leaf substance," and that "salt solution that is formed changes the colloidal state of the chlorophyll in the chloroplasts and makes it easily soluble."

MATERIALS AND METHODS

To study the permeability of plant tissues to various organic solvents, seeds of sweet corn, soybean, and garden pea were used. The sweet corn was Burpee's Golden Bantam; the soybeans were of the Lincoln variety locally acquired; and the garden pea seeds were Burpee's Blue Bantam (wrinkled). The seeds were soaked in organic solvents at room temperature (25–30° C.), and weighings of the superficially dried seeds were taken at the end of 1 day, 4 days, and 7 days. The organic solvents were methanol, ethanol, and acetone. Methanol was dehydrated with magnesium turnings which forms magnesium methoxide, then refluxed and distilled. Ethanol was dried over Drierite and then with metallic sodium which forms sodium ethoxide; then refluxed and distilled. Acetone was dried merely over Drierite, then refluxed and distilled. The aqueous dilutions of methanol, ethanol, and acetone were made on the basis of volume.

Changes in the lengths of soybean seeds soaking in absolute, 95%, 90%, and 85% methanol were determined by use of a vernier calipers. Specific gravity for some of the solutions was determined by use of a specific gravity bottle.

A Klett Biocolorimeter was used to determine the relative amounts of chloroplast pigments extractable by acetone-water solvent to which $NaNO_3$ and cane sugar, respectively, had been added as compared to the amounts of chlorophyll pigments extractable by acetone-water solvent alone. The amount of pigments extractable by 85% acetone was used as the standard for comparison. It was given an arbitrary value of 100 and all the others were evaluated comparatively by colorimetric measurements.

EXPERIMENTAL RESULTS

Seeds of sweet corn, soybean and wrinkled garden pea soaked in absolute methanol, absolute ethanol, and anhydrous acetone showed a remarkably greater imbibition of absolute methanol during the first day than of absolute ethanol or of anhydrous acetone (Tables I and III). To be specific, soybean seeds absorbed or imbibed absolute methanol equal to 7.7% of their dry weight during the first day, whereas they imbibed absolute ethanol equal to only 1.6% of their dry weight in the same period of time, and anhydrous acetone equal to only 0.1% of their dry weight. This difference is more pronounced at the end of 4 days and at the end of 7 days. The soybean seeds in absolute methanol also showed a distinct increase in

TABLE I

INCREASE IN WEIGHT OF 100 SOYBEAN SEEDS SOAKED IN VARIOUS ORGANIC SOLVENTS

Solvent in Days	Weights in Grams	Per Cent Increase
Absolute methanol (100%)		
0	12.95	....
1	13.95	7.7
4	14.30	10.4
7	14.59	12.7
Absolute ethanol (100%)		
0	14.94	....
1	15.50	1.6
4	15.65	2.6
7	15.77	3.4
Anhydrous acetone (100%)		
0	13.17	....
1	13.18	0.1
4	13.31	1.1
7	13.47	2.3

TABLE II

INCREASE IN LENGTH OF SOYBEAN SEEDS SOAKED IN WATER-FREE SOLVENTS. DATA ARE AVERAGES OF 10 SEEDS. ABSOLUTE INITIAL AVERAGE LENGTHS ARE GIVEN IN PARENTHESES

Days Soaked	Absolute Methanol (.725 cm.)	Absolute Ethanol (.742 cm.)	Anhydrous Acetone (.718 cm.)
1	.036 cm.	.010 cm.	.003 cm.
4	.061 cm.	.020 cm.	.020 cm.
7	.....	.031 cm.	.031 cm.
14	.091 cm.	.039 cm.	.041 cm.

TABLE III

INCREASE IN WEIGHT OF 50 SEEDS OF WRINKLED PEA AND OF 50 SEEDS OF SWEET CORN SOAKED IN VARIOUS ORGANIC SOLVENTS

Solvent in Days	Wrinkled Pea		Sweet Corn	
	Weight in Grams	Per Cent Increase	Weight in Grams	Per Cent Increase
Absolute methanol (100%)				
0	14.30	....	12.61	....
1	15.76	10.2	13.72	8.8
4	16.73	17.0	14.24	12.9
7	17.38	21.5	14.55	15.4
Absolute ethanol (100%)				
0	14.69	....	13.92	....
1	14.89	1.4	14.16	1.7
4	15.42	5.0	14.18	1.9
7	15.83	7.8	14.25	2.4
Anhydrous acetone (100%)				
0	13.03	....	13.46	....
1	13.03	0	13.61	1.1
4	13.00	0	13.59	1.0
7	13.00	0	13.60	1.0

length in contrast to very little change in the length of seeds soaked in absolute ethanol and anhydrous acetone (Table II).

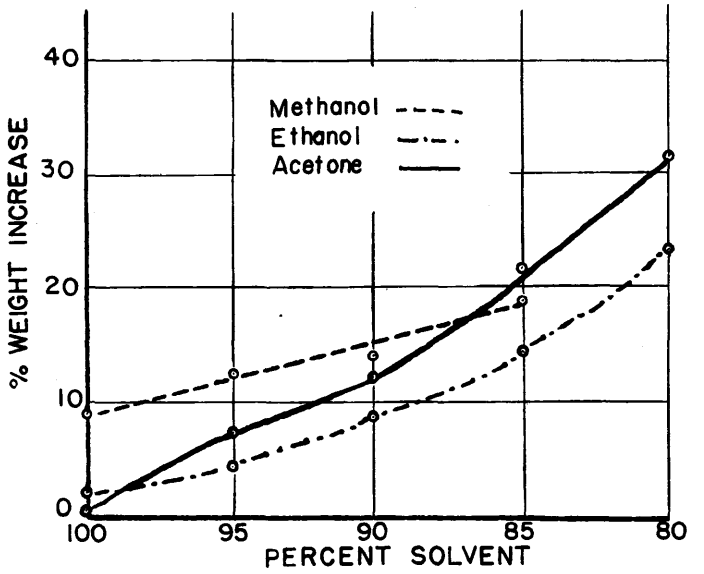

FIG. 1. Per cent increase in weight of sweet corn seeds soaked in various aqueous dilutions of methanol, ethanol, and acetone for one day.

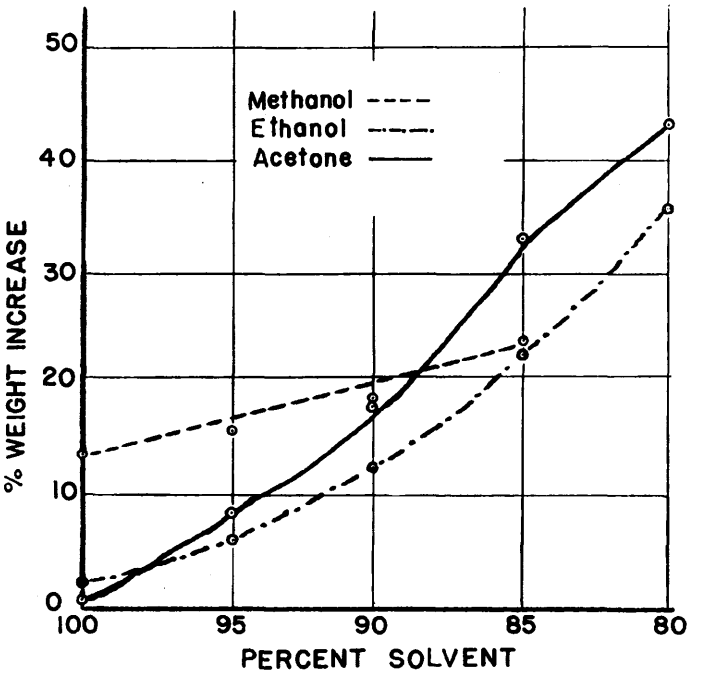

FIG. 2. Per cent increase in weight of sweet corn seeds soaked in various aqueous dilutions of methanol, ethanol, and acetone for four days.

Seeds in absolute methanol get to be quite soft and can be sectioned very easily with a razor blade. This is not true for seeds soaked in absolute ethanol or in anhydrous acetone which only permeates slightly and produces little change in the original hardness of the seeds. The data for sweet corn seeds and for wrinkled pea seeds (Table III) are even more diverse in the differences. Permeability in various aqueous dilutions of methanol, ethanol, and acetone can best be described by the graphic illustrations of Figures 1–3. The points for methanol form an approximate straight line which has about the same slope in the 1-day, 4-day, and 7-day periods. The points on the graph for ethanol and for acetone form curves which have distinctly greater slopes than the line for methanol. Of the curves for ethanol and for acetone, the curve for acetone has the greater slope, and the slope for the 4 days is greater than for one day and greater for 7 days than for 4 days. This means that dilutions of these three organic solvents with water

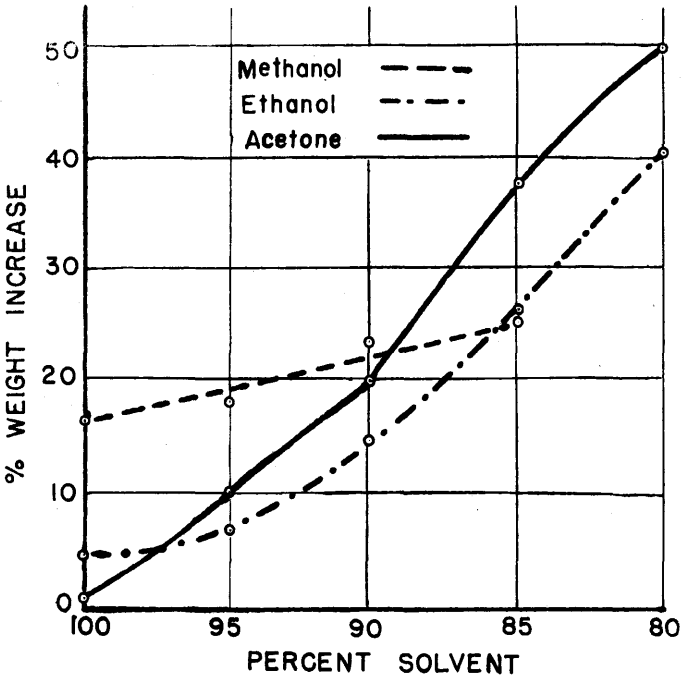

FIG. 3. Per cent increase in weight of sweet corn seeds soaked in various aqueous dilutions of methanol, ethanol, and acetone for seven days.

produce the greatest change in imbibition with acetone, and least with methanol. The result is that all of these lines intersect. Whereas 100% methanol is imbibed far more abundantly than either 100% ethanol or 100% acetone, 85% acetone is imbibed more than either 85% ethanol or 85% methanol. All of the graphs show this tendency for greater increase in absorption of acetone with greater aqueous dilutions and for a rather uniform increase in absorption of methanol with greater aqueous dilutions. Ethanol is intermediate between methanol and acetone.

The specific gravity determinations presented in Table IV show very little difference in values at the start as compared to the end of an imbibitional period. This seems to indicate that there is very little or no differential or preferential

absorption of either the organic solvent or the water which dilutes the organic solvent.

Sugar and Salt Effect.—That penetrability of solvent is a prime factor in the extraction of chloroplast pigments is further substantiated by the effect of salts and sugars. Sodium nitrate decisively increases the amount of chloroplast pigments extracted from dry nettle leaf powder in one hour by 80% acetone, and cane sugar decreases the amount of chloroplast pigments extractable in one hour (Table V). Quantities used in each case were 1 g. of dried nettle leaves, 20 ml. of acetone solvent, 1 g. $NaNO_3$ and 5 g. of cane sugar. Values are those obtained by comparing the extract colorimetrically with the extract in 85% acetone which was used as the standard and arbitrarily given the value of 100.

A corresponding effect is registered by the sodium nitrate and the cane sugar on the initial rate at which seeds imbibe 80% acetone. The data are given in Table VI.

TABLE IV

SPECIFIC GRAVITY CHANGES IN SOLVENTS DURING A 21-DAY SOAKING PROCESS

SOLVENT	SEEDS	SPECIFIC GRAVITY	
		Beginning	End
Acetone, 100%	Wrinkled pea	.790	.789
95%	" "	.807	.807
80%	" "	.826	.822
100%	Sweet corn	.790	.792
95%	" "	.807	.811
90%	" "	.826	.827
80%	" "	.862	.868
Methanol, 100%	Sweet corn	.793	.800
95%	" "	.811	.819
90%	" "	.827	.829
85%	" "	.843	.838
Ethanol, 100%	Sweet corn	.795	.801
95%	" "	.809	.811
90%	" "	.827	.829

Sodium nitrate and cane sugar have another effect, also. This effect is on the solubility of chloroplast pigments. The effect is, however, slight (about 6–9%) and in the direction of decreased solubility. The greatest influence of the salt and the sugar on extractability of chloroplast pigments must be accredited to their effect on the permeability of the plant material to the solvent.

DISCUSSION

The extraction curves of Willstätter (1915) as presented in Figure 4 can be explained by a joint consideration of chloroplast pigment solubility and of the relative permeability of plant tissues to these solvents and their aqueous dilutions. Chlorophyll pigments are always more soluble in water-free fat solvents and become less and less soluble as the water content increases. Although chloroplast pigments are more soluble in anhydrous acetone than in 80% acetone, the dominant

influence in extraction is the very low permeability of dry leaf powder to anhydrous acetone and its extremely high permeability to acetone containing 20% water.

Dry plant tissues are extremely permeable to methanol. The permeability to absolute methanol is high, and the permeability increases only slowly to mixtures of water and methanol. Chloroplast pigment solubility in methanol decreases rather rapidly as the water content increases. With acetone the chloroplast pigment solubility does not drop nearly as rapidly with an increase in water content (Eyster, unpublished data). Since extraction of chloroplast pigments seems to be due to a combined joint influence of solubility and tissue permeability, this perhaps is adequate to account for the fact that extraction of chloroplast pigments is always greatest in absolute methanol and decreases in dilutions of methanol with water.

TABLE V

EXTRACTABILITY OF CHLOROPLAST PIGMENTS FROM DRIED NETTLE LEAVES IN ONE HOUR AT ROOM TEMPERATURE AS AFFECTED BY $NaNO_3$ AND CANE SUGAR. THE DATA ARE ONLY COMPARATIVE VALUES AND WERE OBTAINED BY COLORIMETRICALLY COMPARING THE FILTERED SOLUTIONS WITH THE 85% ACETONE FILTRATE VALUED ARBITRARILY AT 100

Solvent Per Cent	Acetone	Acetone + $NaNO_3$	Acetone + Cane Sugar
100%	24	21	17
95%	67	83	55
90%	92	113	82
85%	100	132	86
80%	94	134	63

TABLE VI

INFLUENCE OF $NaNO_3$ AND SUCROSE ON THE INCREASE IN WEIGHT OF 50 WRINKLED PEA SEEDS SOAKED IN 80% ACETONE

SOLUTION (100 ml.)	WEIGHT IN GRAMS AND PER CENT INCREASE		
	At Start	One-Half Day	One Day
80% acetone	12.62	13.08 (3.7)	14.09 (11.7)
80% acetone + .1 M $NaNO_3$	13.05	14.44 (10.7)	15.25 (16.9)
80% acetone + 10 g. cane sugar	13.01	13.31 (2.3)	13.92 (7.0)

SUMMARY

The data obtained by Willstätter and Stoll (1913) on the amount of chloroplast pigments extractable from dried nettle leaf powder by methanol, ethanol, acetone and their aqueous dilutions seem to be a net result of the permeability of the plant material to the solvents and of the solubility of the chloroplast pigments in the respective solvents. Seeds of sweet corn, soybean, and wrinkled pea were soaked in 100%, 95%, 90%, 85% methanol, in 100%, 95%, 90%, 85%, 80% ethanol, and in 100%, 95%, 90%, 85%, 80% acetone. Weighings were made immediately before soaking and at the end of 1, 4, and 7 days. Length measurements were

also obtained from soybean seeds. The weight increases during the first day for wrinkled pea seeds were 10.2% in absolute methanol, 1.4% in absolute ethanol, and 0% in anhydrous acetone. With aqueous dilutions and also for longer periods of time the increases were greater. The data for soybean seeds and for sweet corn seeds were the same comparatively as for wrinkled pea seeds.

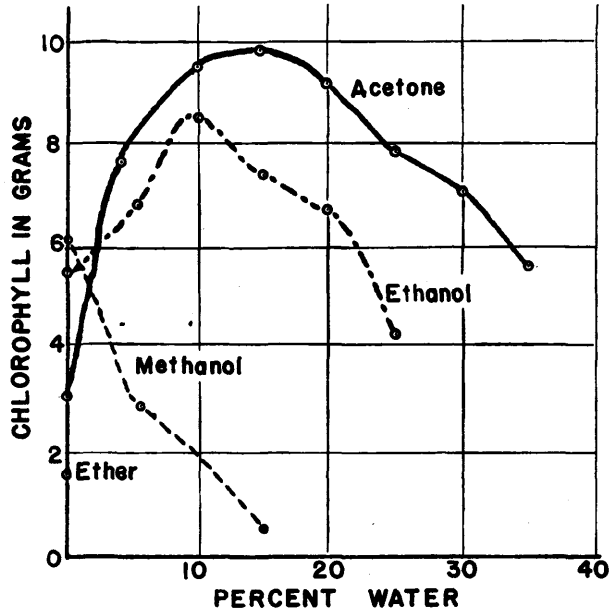

FIG. 4. Extractability of chlorophyll from dried and pulverized leaves by various organic solvents. After Willstätter (1915, p. 335).

Sodium nitrate decisively increases the amount of chloroplast pigments extracted from dry nettle leaf powder in one hour by 80% acetone, and cane sugar decreases the amount. A corresponding effect is registered by the sodium nitrate and the cane sugar on the initial rate at which seeds imbibe 80% acetone.

REFERENCES

Willstätter, R., and **A. Stoll.** 1913. Investigations on chlorophyll. (Authorized English translation by Schertz and Merz. 1928. Science Press. Lancaster, Pennsylvania.)
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