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Phloridzin

I. THE SIGNIFICANCE OF PHLORIDZIN IN APPLE AND PEAR TISSUE.

II. THE HYDROLYSIS AND ESTIMATION OF PHLORIDZIN.

By

E. M. HARVEY

CORVALLIS, OREGON

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Phloridzin

By

E. M. Harvey

I. THE SIGNIFICANCE OF PHLORIDZIN IN APPLE AND PEAR TISSUE

INTRODUCTION

The almost universal presence of glucosides in plants, and their very diverse and often remarkable constituents, should lead one to suppose that a large amount of functional information relative to these substances must have accumulated. Such an assumption, however, does not appear to be borne out by the facts, for the large portion of the knowledge of glucosides is of a purely chemical nature; the physiological role apparently having received comparatively little attention.

The relatively few physiological studies made have already led to the general belief that many glucosides, or their glucosidal derivatives, must play indispensable roles in vital processes. The fact that there is usually associated with every glucoside an enzyme capable of splitting it is in itself rather significant. Notwithstanding these suggestive leads, the data relative to the function of the commonest glucosides remain very meager indeed. This situation may be illustrated simply by turning to a few of our standard reference books dealing entirely, or in part, with this group. In these books, one reads in the single chapter, or sub-chapter, under the heading, "Physiological function of glucosides," the same brief paragraphs, continually copied from one work to another, with but slight alteration.

The interest of the writer in the glucoside, phloridzin, of apple tissue, originated several years ago on the occasion of finding, in connection with other chemical analyses, this substance in abundance in certain apple tissue, and being unable to find in the literature anything regarding its physiological function and almost nothing on its specific distribution. No definite work was inaugurated, however, until 1922, following the appearance of a certain paper by Mitra.[9] Since that time the writer has published two papers,[6, 7] containing data on the behavior of phloridzin. The present work is an extension into the same general field.

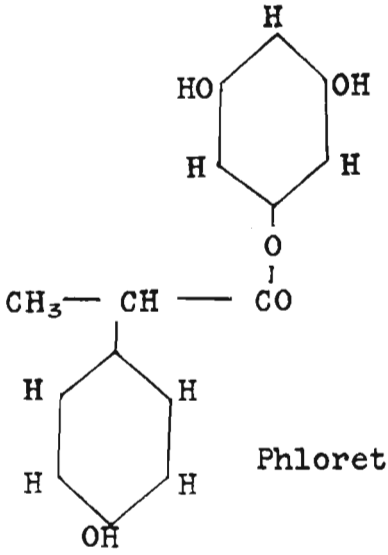
But before proceeding with the report, a few general remarks about the phloridzin itself might be of interest. According to Wehmer,[10] phloridzin is found principally in the apple and pear tree, but it has been identified also in several other Rosaceae, such as plum, sweet cherry, and probably Spirea, Crataegus and Amelanchier. In so far as can be judged from the information at hand, phloridzin is the dominating glucoside only in the apple and pear tree. Even in the latter, arbutin apparently occupies an important secondary position.

As to the chemical composition, the empirical formula of phloridzin and its first hydroletic products are as follows:

$$C_{21}H_{24}O_{10} + H_2O = C_6H_{12}O_6 + C_{15}H_{14}O_5$$

phloridzin — glucose — phloretin

Of the two primary hydroletic products, the most interesting, and probably most significant is phloretin, which itself contains two benzine rings, one of which is phloroglucine, the other possibly hydroquinone.



Phloretin

Phloroglucin has been identified in the free condition in apple bark; and likewise both phloroglucin and hydroquinone have been found in the pear. Hydroquinone is rather abundant in the latter, where it is associated with its own derivative arbutin. Some of the interrelations in the tissue of arbutin and hydroquinone have been tolerably well worked out. (See Czapek.[3]) That substances of such interesting constitutions, and perhaps high potential for influencing vital processes, occur free, or in loose combination with glucose in living tissue, should be regarded as a fact worthy of serious consideration. It seems remarkable that there is so little available information on the physiological functions of the common phenol acids and the glucosides derived from them.

The present work represents a temporary yielding to a curiosity generated through reflection on the above situation, the curiosity being self-justified by the apparent close relation of this subject to the general physiological studies on fruit trees already in hand.

The specific aim of the work was to determine the relation of phloridzin to the rate of growth of shoots, and to study further the distribution of phloridzin within the shoots.

MATERIAL AND METHOD

The work here recorded was done with apple and pear shoots. The apple was represented by three varieties, Jonathan, Wagener, and Arkansas Black; and the pear by two varieties, Glout Morceau and Bartlett. The shoots were measured, and samples taken for analysis at intervals through the summer, beginning at a very early stage of their development.

Collection and preservation. All shoots collected for analysis were gathered in the morning before 8:00 a.m., and the leaves were removed immediately to check loss of water. They were then placed in tightly closed cans and brought into the laboratory for separation into "tip," "middle," and "basal" portions, and determination of fresh weights. The entire time from beginning of each collection to completion of fresh weight determinations was always somewhat less than one hour. The tissue was then placed in bottles; covered with 90 percent alcohol and heated in a water bath for one hour at 70° C. The fresh weights of samples ranged from 20 to 50 grams.

Dry weight. When ready to begin analysis, the entire sample was transferred from the bottle to a 10-centimeter evaporating dish, a little hot water being used to aid in the transference. After reduction to "moist-dryness" on steam bath, the dish was placed in vacuum oven and contents dried to constant weight at 85° C. and pressure of 7 to 10 cm. of mercury. The dry weight of the entire sample was then recorded.

Preparation for extraction. The entire dried sample was ground to a fine powder in a mill and transferred to a glass-stoppered bottle. Thence samples of 3 to 9 grams were carefully weighed out for extraction.

Extraction. The purpose of the extraction was to remove sugars and phloridzin. Either alcohol or hot water might have been employed as solvent, but the latter was adopted for this work. It was found by experience that water 50° to 60° C. was sufficiently "hot" for complete extraction of phloridzin. Each extraction apparatus used was constructed as follows: An opening was blown into the bottom of an ordinary test tube of 2 cm. diameter. The opening at bottom was covered, from the inside, by means of a thimble of copper milk-strainer cloth, and the thimble was filled with a plug of glass wool. This, the extraction tube, was lowered into a closely fitting and packed spiral of copper tubing. During extraction, a continuous stream of water at approximately 65° C. was passed through this coil. The test tube itself was connected at top with a glass tube and rubber stopper, so that hot water (60° to 70° C.) for extraction could be forced through the tissue at a slow and steady rate. The water bearing the extractives dripped from the bottom of the tube into a funnel and 250 c.c. volumetric flask.

The procedure adopted was to transfer the weighed dried sample to a small beaker and there thoroughly saturate it with water to allow opportunity for complete swelling before transferring it to the extraction tube. After transferring the wet tissue to the extraction tube, the apparatus was connected up as indicated. About 230 c.c. water was passed steadily through the tissue, at such a rate that the extraction consumed about 1½ hours.

Total sugar and phloridzin. The entire extract was partly cleared with neutral lead acetate and the excess lead precipitated with sodium carbonate. The final volume of extracts became 250 c.c. It should be mentioned that phloridzin is almost insoluble in pure cold water, but if once brought into solution in these apple extracts, containing many organic impurities, it is prevented from crystallizing out, although it may be present in considerable quantities. If solutions are left standing after completion of the clearing procedure, however, phloridzin is very likely to begin to crystallize. In order to avoid this difficulty, the clearing and division of sample for total sugar and phloridzin determinations were carried through as rapidly as possible.

Total sugars. A 75 c.c. portion was taken for total sugars, using the official inversion procedure, after which determination of the sugars was made as described in Bulletin 200 of this Station.

Phloridzin. A 75 c.c. portion was pipetted into a 250 c.c. Florence flask, the procedure being exactly the same as that described in Bulletin 200, except that the hydrolysis period was changed from 2½ hours to fifteen minutes. The reason for the alteration will be given in Part II of this report.

EXPERIMENTAL

Certain data published in Station Bulletin 200 showed that not only is phloridzin more abundant at the apex of the shoot, but also during the most actively growing period it decreases there less rapidly than in other portions of the shoot. Thus the data indicated that phloridzin is closely associated with the regions of high metabolic activity; in fact, the possibility of the quantity of phloridzin present being taken as a measure of the degree of that activity suggested itself. In such case, a curve representing the phloridzin content of an organ should parallel a corresponding curve of metabolic activity. Accordingly, in a growing shoot, and especially the tip portion, the phloridzin and the rate of growth curves might be expected to show a general similarity. This latter situation had been suggested from previous findings, and it became the chief aim of the present work to determine the relationship between phloridzin and growth, in so far as observation on the rate of elongation of shoots and variations in their phloridzin content might permit. The results of such observations are presented below.

Apple. Shoot-growth records for the apple are given in Table I, but the greater interest will be found in the graphs of growth rate constructed from these data, and superimposed upon the corresponding graphs representing variations of phloridzin in the respective varieties. These graphs are given in Figs. 1, 2, 3. Arkansas Black shoots show plainly a slower rate of growth at the beginning of the season. This slower initial growth rate is rather difficult to demonstrate, and in the present observations a particular effort was made to do so. Unfortunately in this respect, the attempt was a failure for Wagener and Jonathan. Perhaps the observations were too infrequent or they were not begun early enough in the season. In the Jonathan, however, the slower initial rate is apparently very nearly indicated.

The chemical analyses of the apple shoots are presented in Tables III-V, and the graphs representing seasonal phloridzin variations are shown in Figs. 1-3 as previously mentioned. The chemical data show that water and phloridzin and usually total sugars are most abundant in the tip, and least so at the base of the shoots at all times during the

TABLE I. GROWTH OF APPLE SHOOTS, SUMMER 1924.

(Each value the average of 35 to 60 shoots.)

Arkansas Black	Date	May 7	May 13	May 19	June 2	June 25	September 15
	Length of shoots, *cm.*	3.1	8.5	15.7	30.4	35.9	36.7
	Rate per day, *cm.*	.....	0.91	1.20	1.05	0.24	0.01
Wagener	Date	May 8	May 15	May 28	June 11	July 7	August 10
	Length of shoots, *cm.*	4.4	8.3	15.3	22.3	31.9	33.2
	Rate per day, *cm.*	.....	0.56	0.54	0.50	0.32	0.04
Jonathan	Date	May 6	May 14	May 26	June 17	.....	September 19
	Length of shoots, *cm.*	5.3	12.7	23.6	29.2	.....	32.0
	Rate per day, *cm.*	.....	0.92	0.91	0.30	.....	0.03

TABLE II. CHEMICAL ANALYSES OF ARKANSAS BLACK SHOOTS

Date	Part of shoot analyzed	Water	Total sugars*	Phloridzin*
		%	%	%
May 7	Tip	76.2	2.82	7.0
May 19	Tip	74.5	3.75	9.7
	Base	71.2	3.70	8.0
June 2	Tip	73.5	4.08	14.1
	Middle	70.3	2.32	7.1
	Base	65.2	3.12	7.2
June 25	Tip	70.0	3.50	12.5
	Middle	63.6	2.91	7.8
	Base	60.2	3.02	7.0
Sept. 15	Tip	50.4	3.13	7.7
	Middle	49.6	3.05	5.1
	Base	46.3	2.13	3.8

*Sugars and phloridzin values on dry weight basis in this and the following chemical tables.

TABLE III. CHEMICAL ANALYSES OF WAGENER SHOOTS

Date	Part of shoot analyzed	Water	Total sugars	Phloridzin
		%	%	%
May 15	Tip	75.7	3.28	6.84
	Base	72.6	2.83	5.93
May 28	Tip	73.1	4.15	8.48
	Middle	69.2	3.21	8.65
	Base	67.4	2.42	4.09
June 24	Tip	69.7	3.56	12.20
	Middle	63.2	2.97	6.11
	Base	57.0	2.84	4.73
Sept. 19	Tip	50.4	2.54	6.73
	Middle	48.5	2.59	4.63
	Base	45.2	2.19	3.85

TABLE IV. CHEMICAL ANALYSES OF JONATHAN SHOOTS

Date	Part of shoot analyzed	Water	Total sugars	Phloridzin
		%	%	%
May 14	Tip	73.4	2.53	6.80
	Base	72.1	3.07	6.60
May 26	Tip	73.4	2.92	12.52
	Middle	70.4	2.93	7.21
	Base	66.2	2.87	6.28
June 17	Tip	69.3	3.13	11.13
	Middle	68.7	2.37	6.68
	Base	59.3	2.50	5.15
Sept. 19	Tip	48.6	2.39	5.85
	Middle	47.2	2.56	4.37
	Base	45.3	2.34	3.32

growing season; a situation in agreement with the writer's previous findings. The most interesting portion of the shoot is the "tip," since this includes the most active tissues; namely, the growing point and later the developing bud. In the tip of the shoot, phloridzin increases for a time, and then gradually diminishes to the end of the summer. This phloridzin maximum does not exactly coincide in point of time with the growth rate maximum. That is to say, they do not coincide in Arkansas Black shoots, and it is not likely that they would have coincided in Jonathan and Wagener had the characteristic type of growth maxima been demonstrated. The phloridzin maximum lags about two weeks behind the growth rate maximum. Also the growth curve falls much more rapidly than the phloridzin curve. These points will be mentioned again farther on in the discussion.

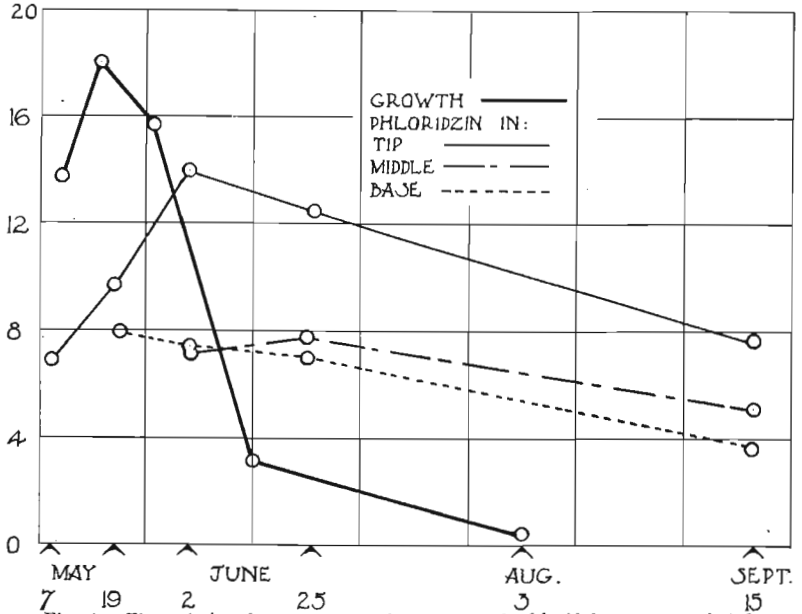


Fig. 1. The relation between rate of growth and phloridzin content of Arkansas Black shoots. Growth rate is in terms of centimeters per day, but multiplied here by 15 to make numerically equivalent to phloridzin percentages.

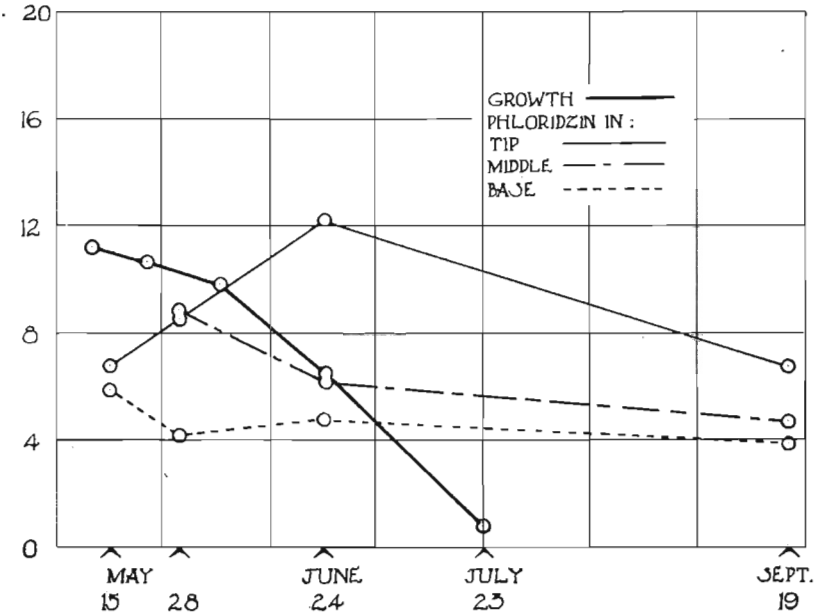


Fig. 2. The relation between rate of growth and phloridzin content of Wagener shoots. Growth rate multiplied by 20. (See legend to Fig. 1.)

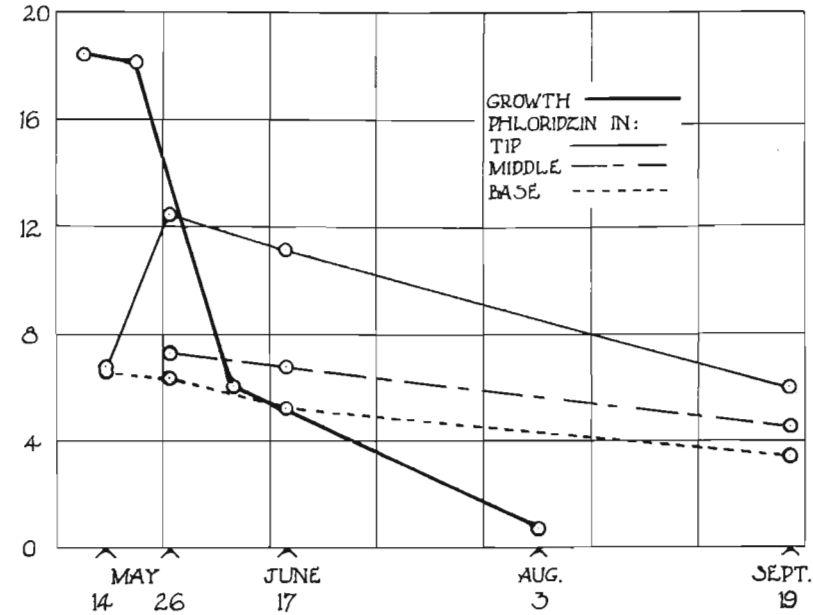


Fig. 3. The relation between rate of growth and phloridzin content of Jonathan shoots. Growth rate multiplied by 20. (See legend to Fig. 1.)

It is interesting in connection with the phloridzin maximum so plainly shown in the present analyses, that this maximum escaped notice in previous work, probably owing to the lateness of the season when the first analyses were done. Nevertheless, a small phloridzin maximum was shown for Grimes (see Bulletin 200, p. 17, Fig. 5), where it was interpreted merely as an experimental anomaly. This illustrates the relative rapidity of the chemical changes taking place in fruit trees early in the spring, and how easily important processes may accordingly be overlooked.

It will be noted in Table I and Figs. 1-3 that the middle and basal portions of the shoot do not show any phloridzin maxima, but instead there is a regular falling of phloridzin content through the season.

TABLE V. GROWTH OF PEAR SHOOTS, SUMMER 1924
(Each value the average of about 50 shoots)

Glout Morceau	Date	April 19	April 25	May 5	May 27	June 23	Sept. 9
	Length of shoots, *cm.*	2.8	5.2	12.4	20.4	23.3	24.9
	Rate per day, *cm.*	.....	0.40	0.72	0.32	0.11	0.02
Bartlett	Date	.....	May 12	May 22	June 4	June 24	Sept. 9
	Length of shoots, *cm.*	.....	5.2	14.3	26.9	33.7	35.1
	Rate per day, *cm.*	.....	.....	0.90	0.97	0.34	0.02

TABLE VI. CHEMICAL ANALYSES OF GLOUT MORCEAU SHOOTS

Date	Part of shoot analyzed	Water	Total sugars	Phloridzin
		%	%	%
April 19	Tip	80.0	3.94	14.2
April 28	Tip	79.6	3.03	16.5
	Base	79.4	2.82	14.4
May 9	Tip	75.2	3.93	19.0
	Middle	74.7	2.35	16.8
	Base	73.3	2.32	14.2
May 27	Tip	68.1	2.60	17.1
	Middle	67.0	2.15	13.7
	Base	66.5	2.50	8.8
June 23	Tip	63.1	2.54	15.2
	Middle	59.3	2.39	7.8
	Base	58.8	2.52	6.9
Sept. 9	Tip	53.2	3.21	9.2
	Middle	50.6	2.65	6.4
	Base	50.3	2.52	5.7

TABLE VII. CHEMICAL ANALYSES OF BARTLETT SHOOTS

Date	Part of shoot analyzed	Water	Total sugars	Phloridzin
		%	%	%
May 12	Tip	78.1	3.63	14.2
	Base	77.0	2.23	13.6
May 22	Tip	75.3	5.13	18.4
	Middle	75.1	2.77	15.7
	Base	74.7	2.13	12.2
June 4	Tip	72.3	3.85	20.3
	Middle	63.8	2.21	16.4
	Base	60.1	2.85	10.4
Sept. 9	Tip	54.1	2.13	9.0
	Middle	50.4	2.07	6.9
	Base	48.8	1.89	5.2

There are no maxima shown by any of the water content curves, but the total sugar curves for the tip of shoots usually show small maxima which agree with those of phloridzin.

Pear. Growth records for the two varieties of pear shoots are given in Table II. Both varieties show the slower initial growth rates followed by maxima, which appear in Glout Morceau on May 5, and Bartlett on May 27. The time difference between the appearance of these maxima is due to the fact that the Glout Morceau began growth at least two weeks ahead of Bartlett, although the Bartlett afterwards grew considerably faster than the Glout Morceau. The graphs representing growth are shown in the phloridzin curves in Figs. 4 and 5.

The chemical analyses of the pear shoots show the same general situation as recorded for apple (see tables VI and VII). Pear shoots contain more water, slightly more total sugar, and about twice as much phloridzin as the apple, but their interrelations are practically the same. Phloridzin maxima in the tips are particularly evident early in the growing period. There is also a slight maximum shown for the "middle" region of the Bartlett shoots on June 4. The growth maximum precedes the phloridzin maximum by about one week, and, clearly, there is also a very rapid falling off of growth as compared with phloridzin.

It should be mentioned in regard to the phloridzin figures for pear tissue, that they must be accepted with reservations. Phloridzin,* probably the dominating glucoside in this tissue also, is accompanied by considerable quantities of arbutin. Both these glucosides contain hydroquinone or hydroquinonelike constituents, and they possess other similar physical and chemical properties, so that a separation is rendered difficult. The quantitative method now being employed for phloridzin will, undoubtedly, cause any arbutin present to be broken down and to appear as phloridzin. If this qualification of the phloridzin data for the pear is kept in mind, they are scarcely less interesting, seeing that the vitiation is due to a similar and closely related glucoside.

DISCUSSION

Many possible functions of glucosides have been suggested. Seeing the great diversity of substances included in this group, wide differences in function might well be expected. Among the different roles assigned to glucosides by various authors may be mentioned the following:

1. As reserve food material.
2. Putting out of action certain harmful by-products of metabolism.
3. Protecting of certain substances, important to metabolism, from too rapid oxidation by tying them up with glucose.
4. As antiseptic and protective agents.

*Since this manuscript was in press, J. P. Bennett and F. B. Lincoln have reported before the Botanical Society at the ninth annual meeting of the Pacific Division of the A. A. A. S. in Portland, that their findings indicate that arbutin occurs to the complete exclusion of phloridzin in the pear tree. If this be true, all phloridzin figures for the pear in this bulletin may be for arbutin. In such case also they are uniformly too high, because the factor for arbutin would be smaller than for phloridzin.

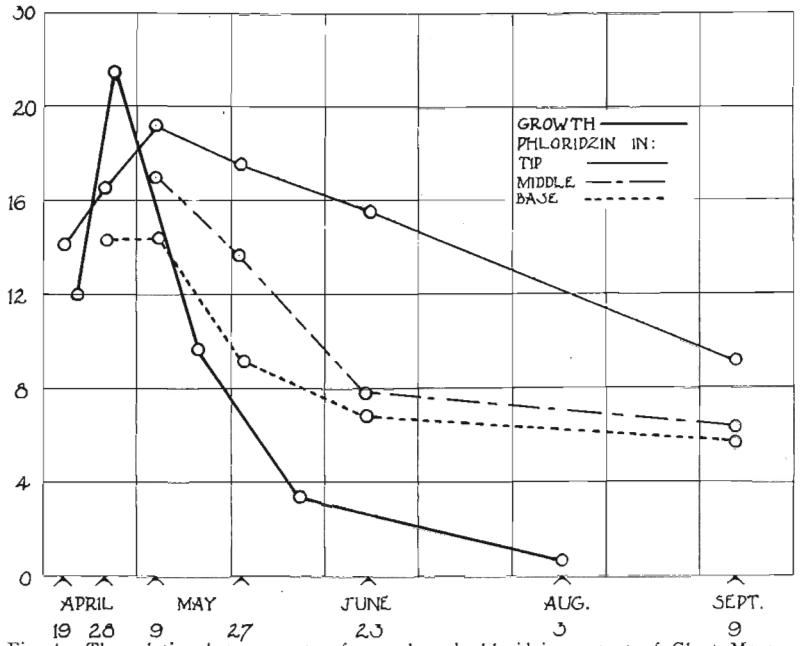


Fig. 4. The relation between rate of growth and phloridzin content of Glout Morceau shoots. Growth rate multiplied by 30. (See legend to Fig. 1.)

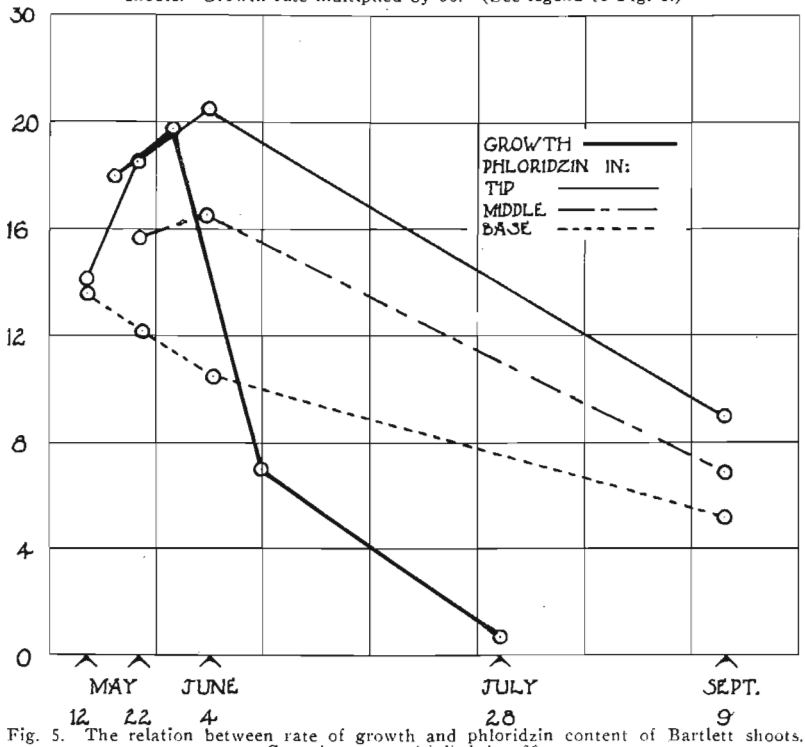


Fig. 5. The relation between rate of growth and phloridzin content of Bartlett shoots. Growth rate multiplied by 20.

5. As activators of growth (harmones) or

6. As accelerators of metabolic processes, particularly that of respiration.

The foregoing list of functions is of such physiological breadth that one may safely assume the function of phloridzin in apple and pear to be included. Although the evidence at hand on phloridzin is meagre, it seems worth while to examine the situation, in an endeavor to determine which of the above functions is being performed.

Phloridzin is most abundant during the season of greatest metabolic activity, that is to say, early summer. This has been shown by Mitra,* who made observations through an entire year. The same situation was indicated in the present report. It was shown also that phloridzin is most abundant at the apex of a growing shoot where growth activity is most rapid. Hence one may assume that phloridzin is closely associated, both with the season of most active growth and with the centers of highest metabolism. This conclusion is in agreement with a general statement concerning glucosides made by Haas and Hill (p. 172).[5] The concentration of phloridzin in the tip region of the shoot appears to be correlated somewhat with the rate of growth. Two points of divergence, however, were noted; first, the phloridzin maximum lags one to two weeks behind the growth maximum; and second, the growth rate falls much more rapidly than the phloridzin content. The above findings seem to point to the following possible roles for phloridzin: (a) as protection against certain phenol acids, originating as by-products of metabolism, or (b) as protection of these phenol acids from too rapid destruction. In the latter case the plant would gain both a storage of them for future use and a means for regulating metabolism. These functions may be practically equivalent. At any rate the role of phloridzin does not appear to be primarily an accelerator of summer growth. For if phloridzin originates as a by-product of active metabolism, and is not an accelerator of it, one must expect the phloridzin curve to lag behind the growth curve; seeing that the phloridzin content at any moment would be the resultant of a variable rate of synthesis and a rather constant rate of translocation or destruction. This lag, however, may be more apparent than real, because other metabolic processes are going on at the tip of a shoot, some of which may be nearly as energy-consuming as linear extension. It is still possible, therefore, that the phloridzin curve is closely indicative of the total metabolic activities going on at the tip of the shoot. Very little seems to be known concerning the relative energy requirements for cell differentiation, bud formation, and other processes.

The lack of phloridzin maxima, within the observation period, in any part of the growing shoot other than the tip, indicated that, where there is no unusual metabolic activity, the tissues are steadily cleared of phloridzin until the minimum percentage of the dormant season is reached.

There is no reason to believe that growth activity is the only one capable of accounting for phloridzin. It was shown by the writer that if apple shoots are ringed and defoliated, the portion above the ring uses up its carbohydrates very rapidly, and concomitantly there is a rapid increase in phloridzin. (See Bulletin 200, p. 31, Fig. 15.) In such

*_Loc. cit._ Mitra's "Maltose" figures equal phloridzin (see Harvey[6]).

shoots there was neither growth nor photosynthesis, hence, if phloridzin synthesis is related to metabolic activity, some abnormally high rate of respiration in these treated shoots must have been responsible for the recorded increase in phloridzin.

Another point perhaps worthy of consideration in connection with the production of phloridzin, or more particularly, the phenol acids upon which the synthesis of phloridzin must depend, is one coming out of Baly's[1, 2] brilliant studies on photosynthesis. Baly* suggested that the first six-carbon product of photosynthesis would appear in the benzine ring form. If so, he tentatively suggested further, that the next stop could be any one of four, which would result respectively in glucose, fructose, inosite, or phloroglucin. Thus comes a possible explanation for the origin of phloroglucin and hydroquinone, the glucosidal constituents of phloridzin. Assuming Baly's specific suggestion to be true, and assuming also that the four substances mentioned were produced normally in some rather definite ratio to one another, then an accumulation of phloroglucin or its derivatives, at the tip of a shoot would not be hard to understand; especially, on recalling that such an area carries on vigorous photosynthesis and is at the same time a greedy consumer of food material. Under these conditions, glucose and fructose would be used up rapidly for energy and tissue building, leaving the other members to become relatively more abundant. Wherever rapid photosynthesis and respiration, or tissue building, occur simultaneously in close proximity, phenol acids might be expected to accumulate.

It must be added that the indirect or protective function of phloridzin, pointed to by the general evidence at hand, does not eliminate the probability of some important direct function, such as a regulator or an accelerator of growth and other processes. Dr. S. M. Zeller of our department of Botany and Plant Pathology, recently brought to the writer's attention some interesting results which he had obtained from the use of phloridzin in cultures of a fungus which causes cankers on apple and pear trees. He noted an increased growth of this organism in the presence of one percent phloridzin. Furthermore, after examining the writer's data relating to the distribution of phloridzin in apple and pear trees, he states that the most rapid growth of the canker coincides with the season of highest phloridzin content; and finally that the pear is more susceptible than the apple† (cf. phloridzin in apple and pear).

*Notes taken by the writer on lecture by Professor Baly before the Oregon Branch of the American Chemical Society, Corvallis, 1924.

†Dr. Zeller has kindly consented to make the following brief statement concerning his growth studies: "The growth of *Nectria galligena* Bres., the fungus causing European canker of apple and pear trees, has been tested out on several liquid, nutrient media in order that media with suitable sources of carbon may be chosen for further experiments relative to the physiology of this organism. In preliminary tests Richard's E solution and potato dicoction, both supplemented with one percent phloridzin, proved to be the most desirable media. Greater mycelial growth resulted where this glucoside, supplementing other sources of carbon, was given, than in the same media where the additional source of carbon was a carbohydrate, as glucose, or an alcohol, as mannite. It is still an open question whether the phloridzin acted merely as a stimulant to the growth of N. galligena or whether it served as an additional source of carbon. On the other hand, however, the presence of the glucoside, salacin, in the media retarded mycelial growth of this fungus. Although the experiments as thus far conducted relative to the effect of phloridzin on the growth of the European canker organism are extremely meagre, they open a field for further investigation and some speculation as to the possible influence of this glucoside on infection and canker enlargement. In correlation with the results of Dr. E. M. Harvey's studies, it is at least interesting to observe: (1) that during the periods of greatest growth rate for pear and apple trees, and also the period of greatest phloridzin content of the bark, the canker makes its most rapid enlargement; (2) that the most common place of infection on a pear or apple stem is also the point of highest phloridzin content; and (3) that in Oregon, at least, the canker is much more prevalent on pear than apple bark, the former having a relatively higher phloridzin content."—S. M. ZELLER.

The chief claim of phloridzin on our interest seems to lie in the fact that a substance having two comparatively loosely tied phenol acid constituents, should be present in very active tissue, sometimes to the extent of 20 percent of the total solids and 5 to 6 percent of the fresh weight; and that the more active the tissues, the greater the quantity of phloridzin present. While no direct function in metabolism is now assumed, the conclusion would seem justified that some important indirect roles are played by it, the most probable of which may be taken either as a protection against any harmful accumulation of phenol acids in active areas, or as a means of temporary storage of such phenols for future use. In the latter case some essential direct service may be performed later by the resulting phloridzin as a regulator or accelerator of vital activity, as suggested by the observation of Dr. Zeller referred to above.

SUMMARY

1. Apple and pear trees contain the greatest quantity of phloridzin in the early summer, coincident with the period of highest metabolic activity. At such times phloridzin may increase to 20 percent of the total solids, or 5 to 6 percent of the fresh weight of the tissue.

2. Pear shoots nearly always contained more water, slightly more total sugars, and about twice as much phloridzin* as the apple. The interrelations of these substances were found to be practically the same in both species.

3. On any given date, phloridzin is most abundant at the tip of the shoot and uniformally less toward the base.

4. In the tip of a shoot, for a relatively short time early in the growing season, phloridzin increases to a maximum, from which point it steadily falls during the remainder of the summer.

5. The "middle" and "basal" portions of a shoot do not show the above type of maximum, for in them phloridzin decreases from the earliest observation to the end of the season.

6. The amount of phloridzin in the tip of a shoot appears to be somewhat correlated with the rate of growth (elongation). The maximum phloridzin content, however, lags one to two weeks behind the apparent maximum growth rate.

7. The observations reported led to the general speculative conclusion that the phenol acids, upon which the synthesis of phloridzin depends, are a sort of by-product of metabolic activity, and that phloridzin serves as a protection against an accumulation of these substances or as a temporary repository of them for future use by the tissue.

8. The possibility of a direct role of phloridzin as an accelerator of vital processes was indicated in a brief reference to some recent, unpublished observations on the effect of phloridzin on growth of fungi by Dr. S. M. Zeller.

*See qualification, p. 13.

II. THE HYDROLYSIS AND ESTIMATION OF PHLORIDZIN

INTRODUCTION

The procedure employed by the writer for the hydrolysis of phloridzin as reported in previous publications[6, 7] was taken over bodily from Darwin and Acton.[4] These authors had said nothing about phloridzin, but recommended the procedure only for the hydrolysis and estimation of maltose in mixtures of other sugars. The writer had occasion to question the accuracy of this method for the determination of maltose in apple tissue, where phloridzin is a normal constituent. On testing the method on pure phloridzin solutions and extracts of apple tissue, it was found to be quite as good a quantitative method for the estimation of phloridzin as for maltose. In fact, it could be used as such in apple tissue, since maltose, if present at all, is in inconsiderable quantities.

The hydrolysis procedure of the Darwin and Acton method requires a so-called "complete inversion" consisting of a 2½ hours' boiling in 2 percent hydrochloric acid.

During the course of numerous analyses, it became apparent that so severe an acid treatment was unnecessary for complete hydrolysis of phloridzin. This led to a special examination of the hydrolysis procedure, for obviously the gentler the hydrolysis could be made, the less would be the destruction of sugars in the mixtures.

EXPERIMENTAL

A. Hydrolysis and estimation of phloridzin in extracts of apple tissue. Some preliminary tests on apple extracts suggested that phloridzin is hydrolyzed very rapidly in boiling 2 percent hydrochloric acid. With this information as a guide, the following experiment was performed: Twenty replicate samples of an apple extract were pipetted into 250 c.c. Florence flasks for hydrolysis. Each of the flasks finally contained: 22 c.c. apple extract; 51 c.c. water, and 4 c.c. concentrated hydrochloric acid (sp. gr. 1.184, giving approximately a 2 percent solution of HCl). It was planned to submit these samples to varying hydrolyzing periods. The hydrolysis was begun by heating the sample over an open flame in such a manner that each came to the boiling point in approximately one minute. After that they were transferred to a Hoskins hotplate and allowed to boil, or simmer, for the hydrolysis period. If that period were longer than 15 minutes, the flask was connected with a reflex condenser. The results of the experiment are given in Table VIII, where the amount of phloridzin was calculated from the increase in reducing power due to the "complete inversion," or hydrolysis, as compared with the reducing power after the official sucrose inversion treatment. It will be noted from Table VIII, and graph I of Fig. 6, that the hydrolysis proceeds extremely rapidly at boiling temperature, so that a maximum value of 58 mg. of phloridzin was reached in 15 minutes. From this time the value falls at such a rate that at the end of the regular 2½ hours' hydrolysis, only 46 mg. of phloridzin was determinable. This fact makes it evident at once that a hydrolysis longer than 15 minutes is undesirable. The sugars are undoubtedly broken down during the long

hydrolysis; particularly one suspects sugars such as fructose, probably present originally in the extract. At any rate an advantage in abandoning the 2½ hours' hydrolysis in favor of a much shorter time is obvious.

B. Hydrolysis of phloridzin in pure solutions. The purpose of the series of experiments under this heading was to observe the relative effects of: (a) length of hydrolysis, (b) quantity of phloridzin, (c)

TABLE VIII. THE INFLUENCE OF TIME ON THE HYDROLYSIS AND ESTIMATION OF PHLORIDZIN IN EXTRACTS OF APPLE TISSUE, WITH BOILING 2 PERCENT HCl

No. of sample	Length of hydrolysis	Phloridzin found	Equivalent percentage in original extract
		mg.	%
1	30 *sec.*	9 (?)	0.15 (?)
2	1 *min.*	18	0.29
3	2 *min.*	32	0.51
4	5 *min.*	51	0.81
5	10 *min.*	57	0.90
6	15 *min.*	**58**	**0.98**
7	30 *min.*	55	0.88
8	1 *hour*	54	0.87
9	1½ *hours*	52	0.84
10	2 *hours*	49	0.78
11	2½ *hours*	**46**	**0.73**
12	3 *hours*	43	0.69

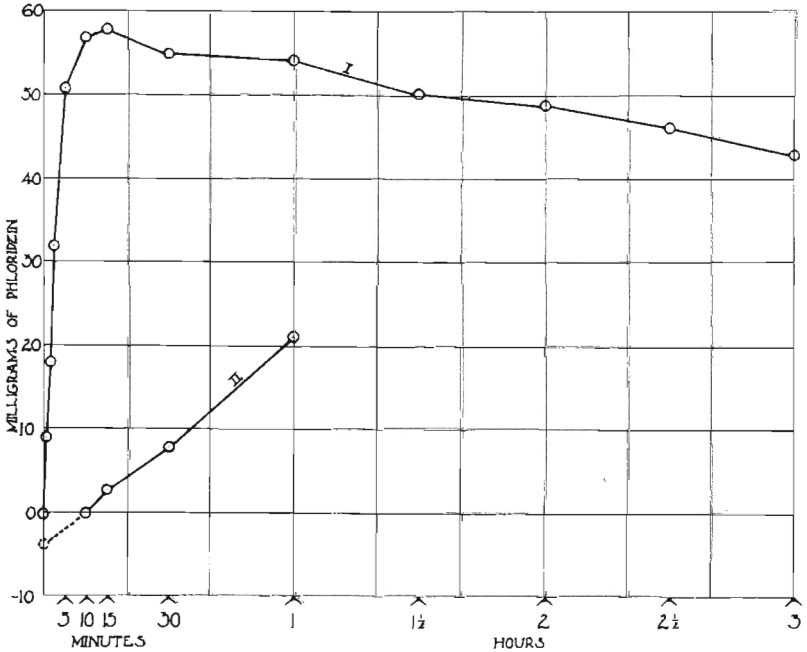


Fig. 6. Hydrolysis of phloridzin in extracts of apple tissue. I. Estimation of phloridzin after hydrolysis with boiling 2 percent HCl. II. The same, but after prolonged (10 minutes plus) exposure to the official inversion solution (i.e., about 2½ percent HCl at 69° C.).

strength of hydrochloric acid, and (d) presence of sucrose (or rather the resulting invert sugar).

The total volume in the hydrolyzing flasks was always 80 cubic centimeters.

A stock phloridzin solution containing 8.673 grams of crystalline phloridzin per litre was prepared. The preceding quantity is equivalent to 8.008 grams anhydrous phloridzin. On account of the insolubility of phloridzin in cold water, the solution was made up to volume at 60° C., and all partitionings of solution were carried out at that temperature.

Experiment I. Hydrolysis of 40.1 mg. phloridzin in 2 percent hydrochloric acid (4 c.c. con. HCl).

The results are recorded in the following table.

Hydrolysis	5 *min.*	15 *min.*	30 *min.*	2½ *hours*
Phloridzin found. *mg.*	38.1	40.1	40.3	38.0

Experiment II. Hydrolysis of 100 mg. phloridzin in 2 percent hydrochloric acid.

Results follow in table.

Hydrolysis	5 *min.*	10 *min.*	15 *min.*	20 *min.*	30 *min.*	2½ *hours*
Phloridzin found *mg.*	88.6	100.0	101.0	99.1	96.7	95.4

Experiment III. Hydrolysis of 150 mg. phloridzin in 2 percent hydrochloric acid.

Results follow in table.

Hydrolysis	5 *min.*	15 *min.*	30 *min.*	2½ *hours*
Phloridzin found *mg.*	122.6	142.7	142.1	134.1

The three preceding experiments indicate that 40 to 100 mg. of pure phloridzin may be completely hydrolyzed and estimated after a 15 minute boiling with 2 percent hydrochloric acid, but that larger quantities are to be avoided.

The difficulty with the large quantities of phloridzin is due perhaps to a greater destruction of the resulting glucose in excessive concentrations.

Experiment IV. Hydrolysis of 100 mg. phloridzin and 1 percent hydrochloric acid (2 c.c. con. HCl).

Results follow in table.

Hydrolysis	5 *min.*	15 *min.*	30 *min.*	2½ *hours*
Phloridzin found *mg.*	54.1	99.0	93.6	91.1

One percent hydrochloric acid proved about as efficient as two percent for a 15-minute hydrolysis.

Experiment V. Hydrolysis of 150 mg. phloridzin in 1 percent hydrochloric acid.

The result of a single observation was as follows: After a 15-minute hydrolysis, only 134.1 mg. phloridzin were found. This suggests that a longer hydrolysis might have been better.

Experiment VI. Hydrolysis of 100 mg. phloridzin with 30 mg. sucrose (equivalent to 31.5 mg. invert sugar) in 2 percent hydrochloric acid.

Results follow in table.

Hydrolysis	5 *min.*	15 *min.*	2½ *hours*
Phloridzin found *mg.*	82.7	95.0	67.1

Experiment VII. As experiment VI except that the concentration of the hydrochloric acid was 1 percent.

Results follow in table.

Hydrolysis	15 *min.*	30 *min.*	2½ *hours*
Phloridzin found *mg.*	93.4	96.7	90.0

Experiments VI and VII show that the presence of invert sugar prevents a complete estimation of phloridzin. The difficulty is due likely to the partial destruction of the sugar, especially fructose, during hydrolysis. One percent hydrochloric acid showed but a doubtful advantage over the 2 percent.

The main conclusion to be drawn from the observations on the hydrolysis of phloridzin both in apple extracts and in pure solutions, with and without sugar, is that the time of hydrolysis with 2 percent hydrochloric acid should be reduced from 2½ hours to 15 minutes. It is possible also that some advantage might accrue from the use of 1 percent of hydrochloric acid and a period of 20 to 30 minutes. However, this latter modification is not strongly recommended. More than a year ago, the writer adopted the 15-minute hydrolysis with 2 percent hydrochloric acid (see Part I) and for the present expects to continue the practice.

C. Hydrolysis of phloridzin by the regular sugar inversion procedure. It is important to know exactly what effect the sucrose inversion has upon phloridzin, because if any is hydrolyzed by this treatment the phloridzin values will be correspondingly lowered. Not only will the phloridzin values be lowered, but the sucrose or total sugars also will be correspondingly raised. Thus an error might be introduced in all total sugar determinations made upon apple and pear tissue, whether or not the investigator be interested in phloridzin.

In the following tests, the inversion procedure examined is the one which calls for a heating for ten minutes in a water bath at 70° C., with 5 c.c. concentrated hydrochloric acid, in a total volume of 80 c.c.[8]

1. *Sucrose only.* Fifty-cubic-centimeter portions of sucrose solution, each containing 30 mg. sucrose (U. S. Bureau of Standards sample) were carried through the above inversion procedure and the invert sugars determined. The sucrose in each inversion was equivalent to 31.6 mg. of invert sugar and the amount found was 31.7 mg. (which was the average of four inversions and eight determinations).

2. *Sucrose and phloridzin.* Three inversions were made where each flask contained 100 mg. phloridzin and 30 mg. sucrose (equivalent to 31.6 mg. invert sugar). The quantity of sugar found was 32.5 mg., thus indicating that nearly 2 percent of the phloridzin present had been hydrolyzed by this inversion treatment.

3. *Inversion of apple extracts.* In this experiment with apple extract, the time of heating was varied. The extract and the amount used in each sample was the same as previously used in the experiment described under division A above. The time of treating varied from that of the regular period (ten minutes) to one hour.

The results are presented in the table following:

Length of "inversion"		10 *min.*	15 *min.*	30 *min.*	60 *min.*
Total sugar found, calculated as glucose *mg.*	.....	14.9	16.1	18.3	23.6
Increase after 10 minutes *mg.*	.....	0	1.2	3.4	8.7
Increase after 10 minutes as phloridzin *mg.*	—4.0*	0	2.9	8.2	21.1

*This value is interpolated from graph II, Fig. 6, and represents, with its sign changed, the phloridzin hydrolyzed by the 10-minute inversion treatment.

The above results show that at the temperature and acid concentration of the regular inversion procedure, phloridzin is gradually hydrolyzed. By referring to graph II, Fig. 6, it will be seen that in one hour 21.1 mg. of phloridzin were broken down. This was about 36 percent of the total 58 mg. previously found (cf. graph I, Fig. 6). Now if graph II is extended downward and back to the axis of ordinates, guided by the general trend of the line, it will strike the axis at a point representing

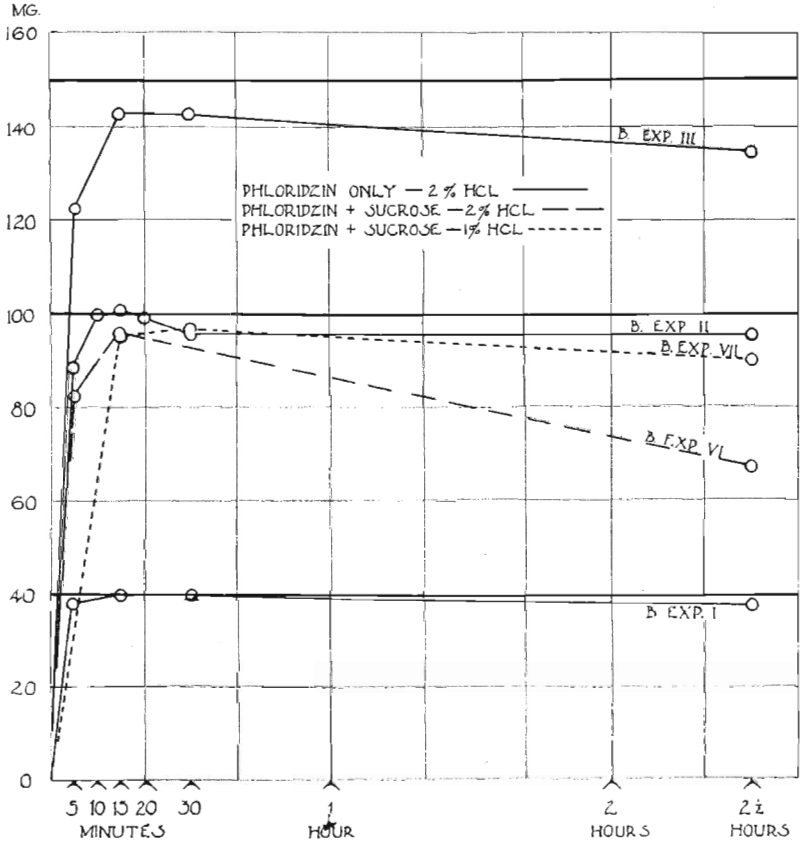


Fig. 7. Graphs showing the hydrolysis and estimation of 40,100 and 140 mg. phloridzin under different conditions.

about 4 mg. phloridzin, the quantity probably hydrolyzed during the official 10-minute inversion period. If this deduction is correct, the apple extract under observation actually contained nearer 62 mg. of phloridzin than the 58 mg. recorded; that is to say, the inversion treatment introduced an error in the present case of more than 6 percent. While the writer is inclined to believe this estimated error too large, the experiment nevertheless strongly indicates that an error is always introduced into phloridzin determination by the inversion procedure, although in any given series it may be fairly allowed for. But the above situation has a

greater significance in connection with the determining of total sugars in apple and pear tissue. For it means that some phloridzin is being hydrolyzed during the inversion treatment, accordingly augmenting the total sugar values. Also until the behavior of arbutin is better known, an additional caution, for a similar reason, should be exercised when determining total sugars, etc., in pear tissue.

Other similar methods of inversion such as the same at room temperature for 24 hours, or with citric acid, might better avoid the hydrolysis of phloridzin. Certainly inversion by invertase solution could be expected to eliminate any error of this kind, either for total sugar or phloridzin estimations.

CONCLUSIONS

1. In the estimation of phloridzin, it should be arranged that the quantity hydrolyzed will be within a range of 40 to 100 milligrams.

2. The time of hydrolysis with 2 percent hydrochloric acid should be reduced from 2½ hours to 15 minutes. But with 1 percent hydrochloric the hydrolysis should be 20 to 30 minutes. The weaker acid, however, is not definitely recommended.

3. The inversion procedure most frequently employed (i. e., 10 minute treatment at 70° C. with 5 c.c. con. HCl) introduces an error into both total sugars and phloridzin determination in apple and pear tissue owing to the slight hydrolysis of phloridzin. Inversion by an invertase solution would therefore appear on *a priori* grounds to be the better procedure in dealing with apple and pear tissues. In any case using the regular inversion, great care should be exercised to keep the condition extremely uniform throughout any given series of analyses.

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