XXXIV. THE EFFECTS OF PHLORIDZIN AND OTHER SUBSTANCES ON FERMENTATIONS BY YEAST.

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INTRODUCTION.

THIS paper deals with a systematic investigation of the effects of a number of glucosides and polyhydric phenols on yeast fermentations.

The experiments to be described were originally undertaken to ascertain whether certain unsaturated substances such as allyl alcohol and acrylic acid, which have powerful effects on bacteria [Quastel and Wooldridge, 1927], have any action on the fermentative activity of yeast. By making use of an apparatus which is capable of yielding very accurate and reproducible results it was found that both these unsaturated compounds bring about an almost complete cessation of the fermentation of glucose by yeast and zymin. A number of other substances were then tested, among them a series of glucosides. Of the glucosides tried one only, phloridzin, showed a marked retarding action on the fermentation of glucose by zymin. Out of a series of polyhydric phenols, phloroglucinol was the most effective in retarding the rate of fermentation by zymin. It is significant that phloroglucinol is the phenol present in phloridzin. Phloridzin, however, had no perceptible action on the fermentation of pyruvic acid by zymin or by live yeast or on the fermentation of glucose by live yeast.

THE APPARATUS.

The apparatus used by us was originally designed by Tryhorn and Jessop in order to measure the rate of decomposition of hydrogen peroxide. It is suitable and extremely convenient for measuring the rate of any reaction involving the evolution of a gas, and also is more accurate than most pieces of apparatus used for the same purpose. As no full description of the device was published by Tryhorn and Jessop [1925] it will be given here. Essentially, it consists of a reaction vessel *A* (Fig. 1) connected through a three-way tap *B* (Fig. 2)¹ with two small gas burettes (*C*₁), each of which can be opened to the atmosphere by a simple tap (*D*₁). As will be seen in Fig. 3 the mounting of the apparatus is such as to enable the whole to be immersed in the water of a constant temperature bath. The reaction vessel (Fig. 1) used

¹ Only half of the apparatus is shown, the two burettes being duplicates (see Fig. 3).

has a capacity of 125 cc. At the bottom of the vessel is a drain tube, and through the top is led a stirring rod *F*, the outlet being closed by a mercury seal *M*. A side-tube, *G*, leads to the three-way tap *B* (Fig. 2), and the gas burettes. The third outlet is a broad tube *H*, leading from the side of the vessel near the top, sufficiently long to project above the surface of the water of the thermostat. It is stoppered by inserting into the constriction at its base a glass rod of suitable diameter covered with tightly fitting rubber tubing, as this arrangement decreases the effective volume of the apparatus and therefore increases its accuracy. For the same reason the connecting tubes between the reaction vessel and the burettes are all capillaries. Independently attached to the base is a vertical iron rod holding a long bearing in which runs an axle carrying a small brass wheel. The axle is bored to an internal diameter such that the top of the stirring rod is held in it by friction. It is essential for the

Fig. 1.

Fig. 2.

success of the apparatus that this axle be held in a long bearing to prevent the stirring rod wobbling and breaking the reaction vessel.

Each gas burette is of 5 cc. capacity and is connected at the lower end with a tube (J_1) which is of the same diameter as the narrow end tubes of the burette itself. This tube is open to the atmosphere and enables one to bring the gas within the apparatus to atmospheric pressure with great accuracy on filling or emptying the burettes. The mounting of the burettes is obvious from Fig. 3. Each burette has a zero graduation on the tube just above the bulb and a millimetre glass scale, P_1 , is fastened to the tube below the bulb. The space between the tubes and scale is filled with Canada balsam to facilitate reading the scale. In order to be able to replace the scale in its original position, if it should be moved, the burette tube has a mark etched on it, to which the zero mark on the scale can be adjusted.

As manometric liquid one hundredth normal sulphuric acid saturated with carbon dioxide was used throughout and was found to be quite satisfactory.

No appreciable loss of gas can occur by diffusion through the long tubes of the manometer.

The burettes were calibrated in the usual manner [Treadwell, 1919] between the zero mark and each of three different graduations on the scale. A graph was constructed showing the scale reading corresponding to 5 cc. of gas at N.T.P. at different atmospheric pressures, correction being applied for the vapour pressure of water at 27°.

In all experiments the stirring rod was run at a speed of about 600 revolutions per minute from the thermostat stirrer. This speed is at least four times

Fig. 3.

that necessary to ensure a constant supersaturation of gas in the zymin suspensions used. The thermostat is of such a width that the counterpoises of the manometers hang freely outside it; in all experiments it was kept at 27° by means of a heating bulb controlled by a toluene-mercury thermometer and electrical relay.

Before each experiment the reaction vessel is cleaned by filling with chromic acid cleaning mixture and allowing to stand for at least half an hour; and after washing it out with water the apparatus is placed in the thermostat and allowed two or three minutes to attain a steady temperature. When the reactants have been introduced into the vessel the latter is connected with one of the burettes by means of the three-way tap, and with the tap *D* open the manometer bulb is raised until the meniscus is at the zero mark. After closing the

neck by the stopper the tap *D* is closed and readings commenced. As carbon dioxide is evolved the manometer bulb is lowered so as to keep the pressure inside the apparatus approximately atmospheric until the meniscus reaches the burette scale, when the bulb is lowered until the meniscus in the tube *J* is at the mark corresponding to a corrected volume of 5 cc. When the meniscus in the burette tube has fallen to the same level the three-way tap is quickly turned to connect the reaction vessel with the other burette, which has already been set to zero and disconnected from the atmosphere. The exact time of turning the three-way tap is noted. By proceeding in this manner a series of readings of times and volumes is obtained. Care must be taken that the three-way tap is only turned when the pressure inside the apparatus is equal to that outside.

Fig. 4. Fermentation of glucose by zymin.

As 1 mm. on the scale corresponds to a volume of about $\frac{1}{100}$ cc. the error of reading the volume is less than $\frac{1}{10}\%$.

The greatest error in measuring the time taken to produce 5 cc. CO₂ (*i.e.* to fill each burette) lies in the interval required to note the time at which the 5 cc. mark of one burette is reached, for immediately this is reached a three-way tap must be turned so that gas flows into the second burette. This error may be appreciable with a reaction proceeding very quickly, but if 5 cc. CO₂ are collected in not less than 2 minutes the error is less than 1 %.

A typical curve of the fermentation of glucose by zymin (yeast treated with acetone) is shown in Fig. 4. The curve consists of two distinct parts, (*a*) an initial stage, lasting about 20 minutes, in which the velocity of CO₂ formation is at first slow and then rapid, and (*b*) a stage in which the production of CO₂

proceeds at a steady linear rate for at least 8 hours. The linear part of the curve, if produced, cuts the ordinate (in our experiments) at a point corresponding to about 7 cc. CO_2 . The value of the steady rate is not changed by decreasing the rate of stirring to a fifth of its usual value. The linearity of the second part of the curve is indicated in Fig. 4, where there has been no smoothing of the curve, and in Table I. This table gives results carried out with two specimens of zymine, F and H, and demonstrates the constancy of the quotient CO_2/t , where CO_2 represents the volume of CO_2 formed in time t when the steady state has been reached (*i.e.* after 20 minutes from the commencement of the fermentation). These results are typical for zymine.

Table I. *Rates of fermentation of glucose by zymine. Times taken after evolution of the first 30 cc. CO_2 .*

Zymine F			Zymine H		
t in min.	CO_2	CO_2/t	t in min.	CO_2	CO_2/t
10.4	10	0.96	9	10	1.11
21.1	20	0.95	19.25	20	1.04
62.5	60	0.96	57	60	1.05
—	—	—	96.2	100	1.04
113.25	110	0.97	—	—	—

With live yeast the second stage in the fermentation of glucose is not so markedly linear as with acetone yeast. The quotient CO_2/t increases gradually (see Table II).

Table II. *Rate of fermentation of glucose by yeast. Times taken after evolution of the first 30 cc. CO_2 .*

t	CO_2	CO_2/t
7.15	10	1.40
13.9	20	1.43
20.5	30	1.46
26.9	40	1.48
33.3	50	1.50
39.65	60	1.51
45.5	70	1.54

PREPARATION OF ZYMIN.

A Dutch baker's yeast was used for the whole of our experimental work. The yeast before use was stored in the ice chest. For preparation of the zymine the following procedure was adopted. The yeast was broken up into small lumps or crumbs and left on the bench at room temperature for 24 hours. 100 g. yeast were then stirred into 600 cc. acetone. After stirring well for 10 minutes the acetone was filtered off in a Büchner funnel and the yeast pressed very hard and powdered. The powder was then ground thoroughly for 2 minutes with 200 cc. acetone, filtered and pressed hard. Finally it was ground with 50 cc. ether for 3 minutes, filtered, pressed hard and dried on the Büchner funnel. The powder was spread on filter paper for half an hour at room temperature and then allowed to stand in the hot room (37°) for 24 hours.

The preparation was stored in the ice chest. Experience has shown that the best results are obtained when the yeast has been well pressed and powdered at each stage of the dehydration. If the final preparation appears granular or crumbly and not a fine powder its activity with glucose is remarkably poor. The yield of zymin from 1 kg. of yeast was 240 g.

REPRODUCIBILITY OF RESULTS WITH A GIVEN SPECIMEN OF ZYMIN.

Our method of ascertaining the effects of substances upon the fermentation of glucose consists in adding the substance in question to the reaction vessel when the steady rate of CO_2 production has been progressing for 20 or 30 minutes. The rates of CO_2 evolution before and after the addition are compared. It is not essential therefore that the same specimen of zymin, or zymin of the same activity, should be used in every experiment. A number of experiments has shown that the same percentage inhibition of the rate of fermentation of glucose is produced by the addition of a given quantity of the inhibitor, when the initial steady rate before addition differs in different experiments.

That a given specimen of zymin, however, retains its activity intact for a considerable period is shown in Table III. This table gives results with zymin F, a number of different experiments being carried out with it on each of several days. No particular effort was made to ensure the reproduction of the same value of the steady rate of fermentation in each experiment, and yet it will be observed that these rates do not differ markedly from each other. The table also gives results with zymin M, a more active preparation than zymin F. In this case out of nine different experiments carried out within 4 days the times for the production of 80 cc. CO_2 lie, with one exception, within $\pm 1\frac{1}{4}\%$ of the mean value.

INFLUENCE OF PHLORIDZIN, ETC. ON THE FERMENTATION OF GLUCOSE BY ZYMIN.

Experimental method. For a reasonably fast fermentation a suspension of 8 g. zymin in 40 cc. 10 % glucose solution was used. 30 cc. water containing 4 g. glucose were placed in a conical flask which was placed in the thermostat (27°) until the solution had attained the temperature of the latter. 8 g. zymin were added to the solution, the flask was stoppered and well-shaken, and its contents were poured into the reaction vessel of the apparatus. The flask was washed with 10 cc. water, the washings being poured into the vessel. The latter was stoppered, stirring was commenced, and readings of the time required for the evolution of consecutive volumes of 5 cc. CO_2 were taken. No buffer was used, this being unnecessary, as shown by control experiments. For the exact comparison of the initial steady rates of fermentation in a series of experiments, it is important that the interval of time from the mixing of the zymin with the glucose to the commencement of gas collection should be constant,

and that the glucose solution should be immersed in the thermostat for the same period.

Table III. *Times taken after attainment of steady rate of fermentation.*

Zymin F			Zymin M		
Date	Exp.	Time in min. required to produce 20 cc. CO ₂	Date	Exp.	Time in min. required to produce 80 cc. CO ₂
June 14	1	20.0	Nov. 24	1	34.8
" 14	2	21.6	" 24	2	35.2
" 15	3	23.0	" 24	3	34.4
" 16	4	22.2	" 25	4	35.1
" 16	5	22.4	" 25	5	36.5
" 16	6	21.6	" 27	6	35.2
" 17	7	22.0	" 28	7	35.2
" 17	8	22.0	" 28	8	34.7
" 20	9	23.0	" 28	9	34.8
" 20	10	23.0			
" 21	11	23.2			
" 21	12	24.0

Fig. 5. Effect of phloridzin on zymin fermentation of glucose.

When the rate of CO₂ evolution had become steady for 20 or 30 minutes the reaction vessel was unstoppered, the substance under investigation added, the vessel restoppered and readings resumed. The substance added was usually either dissolved or suspended in water. The time lost by this process of addition was usually less than a minute.

TYPICAL EFFECT OF PHLORIDZIN.

A typical curve illustrating the effect of addition of phloridzin to a glucose fermentation by zymin is shown in Fig. 5. At the arrow phloridzin was added. The dotted line shows the normal course of the fermentation. We have usually found that the course of fermentation after the addition of phloridzin is *linear*, in contrast to the effect of a substance such as allyl alcohol where the rate of evolution of gas after addition falls off quickly. (This may be, however, purely a matter of relative concentrations.)

THE INFLUENCE OF THE MODE OF ADDITION AND THE
AMOUNT OF SUBSTANCE.

In the experiments to be described the amount of substance under investigation added to the reaction vessel was either 10^{-3} or 10^{-4} of a g.-molecule dissolved or suspended in 4 cc. water. In one series of experiments glucosides (10^{-3} g.-mol.) were added dissolved in 2 cc. ethyl alcohol. They all gave inhibitions, phloridzin retarding the initial rate of fermentation over 83 % whilst the others retarded to the extent of 40–50 %. A control experiment, however, showed that 2 cc. ethyl alcohol alone effected a retardation of 41 % (see Table IV). We deemed it advisable therefore not to add alcohol to the system, though it is clear from this experiment that phloridzin exerts a far greater effect than the other glucosides tested.

In considering the effect of alcohol and the linear rate of a normal zymin fermentation it should be remembered that to produce 1 cc. alcohol about 400 cc. CO_2 must be evolved. In our experimental time, a total of not more than 150 cc. CO_2 are evolved, so that sufficient alcohol has not been produced to make a serious alteration. Zeller [1927] has shown that small quantities of alcohol appear to accelerate rather than diminish the rate of fermentation of glucose by living yeast.

Table IV. *Influence of glucosides in alcoholic solution on zymin fermentation of glucose. 10^{-3} g.-mol. glucoside in 2 cc. ethyl alcohol.*

Glucoside	Time in min. to produce 10 cc. CO_2 before addition	Time to pro- duce 10 cc. CO_2 after addition	% retardation of initial rate	Final p_{H} of reaction mixture
Phloridzin	10.4	62	83.3	6.38
Salicin	10.5	19	44.8	6.1
Amygdalin	10.7	20.5	47.8	6.19
Alcohol (control) 2 cc.	11.7	20	41.5	6.0

Table V.

Exp. A. Zymin F			Exp. B. Zymin F			Exp. C. Zymin X		
t	CO_2	CO_2/t	t	CO_2	CO_2/t	t	CO_2	CO_2/t
9.4	10	1.06	10.6	10	0.94	9.1	10	1.1
22.9	20	0.87	22.1	20	0.90	18.9	20	1.06
28.6	25	0.87	27.76	25	0.90	→ 0.05 g. aesculin added		
→ 10^{-3} g.-mol. (0.446 g.) phlo- ridzin added at this point to the reaction vessel			→ 10^{-3} g.-mol. salicin added			10.2	10	0.98
			13.2	10	0.76	20.3	20	0.98
			26.5	20	0.75	→ 0.04 g. phloridzin added		
25.3	10	0.39	33	25	0.76	17.0	10	0.59
52.9	20	0.38				34.5	20	0.58
67.8	25	0.37						

Table V gives a series of results carried out with two preparations of zymin (F and X). The effects of phloridzin and salicin are compared in the case of zymin F. Here the CO_2/t quotient falls from 0.87 to 0.37 by the addition of 10^{-3} g.-mol. phloridzin, whilst the quotient drops from 0.90 to 0.76 for the addition of the same molar quantity of salicin. In the case of zymin X,

0.05 g. aesculin was added (in water) to the reaction vessel when the CO_2/t quotient was 1.06, causing a fall of the quotient to 0.98. This addition was followed, in the same experiment, by the addition of 0.04 g. phloridzin whereon the quotient fell to 0.58.

EXPERIMENTAL RESULTS WITH GLUCOSIDES AND POLYHYDRIC PHENOLS.

Table VI gives a series of results demonstrating the effects of four glucosides, phenol and the polyhydric phenols on the zymin fermentation of glucose. The effects of 10^{-3} g.-mol. of these substances with zymin F and 10^{-4} g.-mol. with zymin M are shown. Phloretin¹ was also tested.

These results clearly indicate that the substances of outstanding inhibiting action are phloridzin, phloretin and phloroglucinol. In small quantities the effects of phloridzin and phloroglucinol relative to those of the other substances are even more marked, phloridzin having the greatest action. Its retardation of the initial rate of CO_2 production, under our experimental conditions, was 31 % for 10^{-4} g.-mol. and 59 % for 10^{-3} g.-mol.

The fermentation mixtures were examined at the termination of the experiments for change in p_H (see Table VI). The final p_H was very nearly the same (about 6.0) in most cases, and it is clear that the change in p_H is not connected with the retardation of the rate of fermentation. In buffered solution phloridzin has the same retarding action.

Table VI. *Times (in minutes) taken after attainment of steady rates.*

Glucoside, etc.	Zymin F. 10^{-3} g.-mol. glucoside, etc. added				Zymin M. 10^{-4} g.-mol. glucoside, etc. added			
	Time to produce 10 cc. CO_2 before addition	Time to produce 10 cc. CO_2 after addition	% re- tardation of initial rate	Final p_H	Time to produce 10 cc. CO_2 before addition	Time to produce 10 cc. CO_2 after addition	% re- tardation of initial rate	
Phloridzin	11.5	28.0	59	6.0	4.5	6.5	31	
Salicin	11.5	13.3	14	6.0	4.8	4.8	0	
Amygdalin	11.6	13.8	16	5.8	4.5	4.5	0	
Aesculin	11.0	15.5	29	6.0	4.5	4.6	2	
Phenol	10.8	15.7	31	6.0	4.55	4.5	0	
Resorcinol	11.5	16.2	29	6.1	—	—	—	
Quinol	11.1	15.0	26	6.1	4.8	4.8	0	
Catechol	11.2	15.5	28	6.0	4.8	4.7	0	
Pyrogallol	10.8	14.2	24	6.7	4.9	4.9	0	
Phloroglucinol	11.0	18.5	41	6.1	5.1	5.4	6	
Phloretin	12.1	20.0	40	—	—	—	—	

INFLUENCE OF PHLORIDZIN, ETC. ON THE FERMENTATION OF PYRUVIC ACID BY ZYMIN.

Experimental method. Conditions, here, differ from those of the glucose fermentation. It is necessary to use a buffer (acetate), as has been described by other workers [*e.g.* Neuberg, 1927], to secure good and reproducible results. The rate of fermentation is not linear. A typical curve is shown in Fig. 5.

¹ It is possible that the sample of phloretin we used contained a trace of phloridzin.

We find a good reaction mixture to consist of 8 g. zymin, 40 cc. water, 5 cc. 10 % pyruvic acid and 5 cc. 2*M* potassium acetate solution. The zymin is well shaken with the water and introduced into the reaction vessel where it is allowed to reach the temperature of the thermostat. The pyruvic acid and acetate buffer, also at the same temperature, are now added and readings commenced.

Since the rate of CO_2 production is not linear the most convenient method of ascertaining the effect of an added substance is to determine the time taken for the evolution of a given volume of CO_2 in the presence and in the absence of the substance in question.

The substance is added to the reaction vessel after the evolution of the first 10 cc. of CO_2 from the zymin-pyruvic acid mixture.

Fig. 6. Fermentation of pyruvic acid by zymin.

Table VII gives the results of three experiments which are typical. Exp. A gives the variation of the quotient CO_2/t with time in a normal fermentation of pyruvic acid by zymin where 5 cc. water were added after the evolution of the first 10 cc. CO_2 . Exp. B gives the values CO_2/t where 10^{-3} g.-mol. phloridzin (suspended in 5 cc. water) was added after the production of the first 10 cc. gas. In Exp. C aesculin (10^{-3} g.-mol.) was substituted for phloridzin. It will be seen that, within experimental error, phloridzin, like aesculin, has *no* perceptible retarding action on the CO_2 production from pyruvic acid. Table VIII shows that under these conditions (in the presence of acetate buffer) phloridzin has a marked effect on the fermentation of glucose by zymin, the CO_2/t quotient falling from 2.1 to 0.86.

Table VII. *Fermentation of pyruvic acid by zymin.*

Exp. A. 5 cc. water added to reaction mixture after first 10 cc. CO ₂ evolved			Exp. B. 10 ⁻³ g.-mol. phloridzin added in 5 cc. water after first 10 cc. CO ₂ evolved			Exp. C. 10 ⁻³ g.-mol. aesculin added in 5 cc. water after first 10 cc. CO ₂ evolved		
<i>t</i>	CO ₂	CO ₂ / <i>t</i>	<i>t</i>	CO ₂	CO ₂ / <i>t</i>	<i>t</i>	CO ₂	CO ₂ / <i>t</i>
3.25	10	3.1	3.9	10	2.6	4.1	10	2.4
9.1	20	2.2	8.8	20	2.3	8.9	20	2.2
14.75	30	2.0	14.4	30	2.1	14.7	30	2.0
18.0	35	1.9	20.9	40	1.9	—	—	—

Table VIII. *Fermentation of glucose by zymin in presence of acetate buffer.*
Times taken after attainment of steady rate.

<i>t</i>	CO ₂	CO ₂ / <i>t</i>
4.8	10	2.1
9.65	20	2.1
14.3	30	2.1
→ 10 ⁻³ g.-mol. phloridzin added in 5 cc. water		
9.16	10	1.1
19.7	20	1.1
31.2	30	0.96
46.4	40	0.86

None of the glucosides or phenols which we have tested has any inhibitory action, within experimental error, on the pyruvic acid fermentation by zymin. Table IX summarises these results.

Table IX. *Fermentation of pyruvic acid by zymin.*

Glucoside, etc. (10 ⁻³ g.-mol.) added in 5 cc. water to reaction mixture, after evolution of first 10 cc. CO ₂	Time in min. to produce 30 cc. CO ₂
Phloridzin	14.4
Amygdalin	13.0
Salicin	13.5
Aesculin	14.7
Phenol	14.2
Resorcinol	15.9
Catechol	15.7
Phloroglucinol	14.7
Pyrogallol	14.7
Water (control) alone	14.7

EXPERIMENTS WITH LIVE YEAST.

150 g. yeast were crumbled and suspended in one litre of water. The suspension was centrifuged, washed three times and finally shaken with 350 cc. water. A good reaction mixture for glucose fermentation consisted of 6 cc. of the yeast suspension, 4 g. glucose and 34 cc. water. A typical curve for the fermentation is shown in the first part of Fig. 7.

Addition of phloridzin has no perceptible effect on the fermentation of glucose by live yeast. This is indicated in Table X, where Exp. A shows the effect of addition of 6 cc. water, after the attainment of a steady rate, and Exp. B the effect of adding 10⁻³ g.-mol. phloridzin suspended in 6 cc. water.

It will be observed that the addition of phloridzin causes no change in the CO_2/t quotient. Table XI summarises results obtained with various additions in the fermentation of glucose by live yeast.

Phloridzin has no perceptible action on the fermentation of pyruvic acid by live yeast.

Table X. *Fermentation of glucose by live yeast. Action of phloridzin.*

Exp. A			Exp. B		
<i>t</i>	CO_2	CO_2/t	<i>t</i>	CO_2	CO_2/t
6.8	10	1.5	7.15	10	1.4
13.4	20	1.5	13.7	20	1.5
20.5	30	1.5	20.2	30	1.5
6 cc. water added to the reaction mixture at this point			10^{-3} g.-mol. phloridzin in 6 cc. of water added		
6.3	10	1.6	7.4	10	1.4
12.9	20	1.6	14.1	20	1.4
19.2	30	1.6	20.7	30	1.4
25.7	40	1.6	27.4	40	1.5

Table XI. *Fermentation of glucose by live yeast.*

Glucoside, etc. added 10^{-4} g.-mol.	Time to produce 40 cc. CO_2 before addition	Time to produce 40 cc. CO_2 after addition
Phloridzin	14.0	14.1
Aesculin	13.8	13.8
Salicin	13.8	13.6
Amygdalin	13.6	13.7
Phloretin	13.6	13.6
Phloroglucinol	13.5	13.8

THE EFFECTS OF ALLYL ALCOHOL, ETC. ON THE FERMENTATION OF GLUCOSE.

Allyl alcohol has a retarding action which increases with time on both zymen and live yeast fermentations of glucose (Fig. 7). Acrylic acid has a similar action but we have observed no perceptible retarding effects with allylamine or allylacetic acid.

Fig. 7. Effect of allyl alcohol on fermentation of glucose by live yeast.

SUMMARY.

1. Phloridzin shows a much higher retarding action on the normal rate of zymine fermentation of glucose than salicin, aesculin or amygdalin, this being especially marked when relatively small quantities of the substances are used. Under our experimental conditions 10^{-4} g.-mol. phloridzin effects a 31 % retardation and 10^{-3} g.-mol. a 59 % retardation of the rate of fermentation due to zymine. Phloretin shows a similar but somewhat less vigorous action than phloridzin. The rate of fermentation after addition of phloridzin is linear.

2. Of the polyhydric phenols, phloroglucinol (which is the phenol present in phloridzin) is the most effective in retarding the rate of fermentation of glucose by zymine.

3. The retarding action of phloridzin is not exhibited in the zymine fermentation of pyruvic acid, or in the fermentations of glucose and pyruvic acid by live yeast.

4. Allyl alcohol and acrylic acid retard the fermentation of glucose by zymine and live yeast, the retardation increasing with time. Neither allylamine nor allylacetic acid has such an action under the conditions of our experiments.

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