CLX. THE SPECIFIC ACTION OF PLANT ENZYMES.

III. THE SPECIFIC CONDITIONS OF ACTION OF LEAF SALICINASES.

BY A. V. BLAGOVESCHENSKI AND N. I. SOSSIEDOV.

From the Laboratory of Plant Physiology, State University, Tashkend;

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WE have investigated the action of air-dried powdered leaves of some species of *Salix* and *Populus* on salicin solutions at various p_H values. The leaves of all the species of *Salix* and *Populus* (except *S. hastata*) were collected at Tashkend, dried rapidly at 25–30° and ground in a mortar to the state of fine powder. 0.2 g. of this powder was mixed with 10 cc. of McIlvain's [1921] buffer solution (citric acid and Na_2HPO_4) and with 5 cc. of 3 % salicin (Kahlbaum) solution. To all experimental flasks toluene was added and they were kept at 30–33°. For control the same conditions, but without salicin, were maintained. The determinations of glucose liberated were made by the Benedict-Lewis picric acid method, as we described in our previous paper [1925]. The p_H values were determined colorimetrically with Clark and Lubs' indicators. The full details are given for Exp. 1, but in all the other experiments only the difference between the amounts of reducing sugar formed in the presence and absence of salicin are quoted under the heading "Change in reducing sugar."

Exp. 1. The leaves of *P. Bachofenii* Wierzb., collected 13. vi. 1926. Duration of hydrolysis 44 hours.

p_H		Reducing sugar			
		Experiment	Control	Change	
Buffer added	At end	mg.	mg.	mg.	%
3.0	3.0	3.5	2.0	1.5	12
3.4	3.6	5.0	2.2	2.8	22
3.8	4.2	6.0	2.0	4.0	31
4.2	4.6	8.5	2.1	6.4	50
4.6	4.9	12.0	3.8	8.2	64
5.0	5.2	14.0	4.3	9.7	76
5.4	5.3	11.5	3.5	8.0	63
5.8	5.8	17.0	4.3	12.7	100
6.2	6.4	15.0	4.3	10.7	84
6.6	6.5	15.0	4.3	10.7	84
7.0	6.9	15.0	4.6	10.4	82
7.4	7.1	13.5	4.6	8.9	70
7.8	7.3	10.5	3.8	6.7	53

Exp. 2. Leaves of *P. nigra* L. var. *pannonica*. Tashkend, 13. vi. 1926. Duration of hydrolysis 47 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.0	3.5	0.6	10	5.8	6.0	3.5	58
3.4	3.7	1.3	2.1	6.2	6.4	3.0	50
3.8	4.1	0.6	10	6.6	6.7	2.8	47
4.2	4.4	1.6	2.7	7.0	6.9	2.5	41
4.6	4.6	4.5	75	7.4	7.3	1.4	23
5.0	5.4	6.0	100	7.8	7.4	0.7	11
5.4	5.8	4.2	70				

Exp. 3. Leaves of *P. candicans*. Tashkend, 13. vi. 1926. Duration of hydrolysis 19 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.2	3.6	0.0	0	5.8	6.0	0.75	50
3.8	4.6	0.1	6	6.2	6.4	1.5	100
4.2	4.9	0.15	10	6.6	6.6	0.8	53
4.6	5.4	0.35	23	7.0	6.9	0.25	17
5.0	5.5	0.5	33	7.4	7.2	0	0
5.4	5.7	0.1	7	7.8	7.4	0	0

Exp. 4. Leaves of *S. babylonica* L. Tashkend, 13. vi. 1926. Duration of hydrolysis 45 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.0	3.0	1.0	6	5.8	5.8	13.0	76
3.4	3.6	1.6	9	6.2	6.2	15.25	90
3.8	4.0	1.8	11	6.6	6.6	17.0	100
4.2	4.3	3.8	22	7.0	7.0	13.0	76
4.6	4.6	9.0	53	7.4	7.2	12.5	73
5.0	5.0	12.5	73	7.8	7.4	9.0	53
5.4	5.4	10.75	63				

Exp. 5. Leaves of *S. oxica*. Tashkend, 13. vi. 1926. Duration of hydrolysis 46 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.0	3.2	0.1	2	5.8	5.8	3.6	61
3.4	3.7	0.5	8	6.2	6.2	4.6	78
3.8	4.2	0.6	10	6.6	6.5	5.2	89
4.2	4.6	1.0	17	7.0	6.9	5.9	100
4.6	4.9	2.7	46	7.4	7.2	3.0	51
5.0	5.0	3.5	60	7.8	7.3	2.3	39
5.4	5.4	3.0	51				

Exp. 6. Leaves of *Salix* sp.? Tashkend, 13. vi. 1926. Duration of hydrolysis 44 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.0	3.2	0.4	4	5.8	5.7	8.5	89
3.4	3.6	0.5	5	6.2	6.0	9.5	100
3.8	4.1	0.9	9	6.6	6.5	8.5	89
4.2	4.5	1.5	16	7.0	6.9	8.4	88
4.6	4.9	2.6	27	7.4	7.2	3.8	40
5.0	5.2	3.1	32	7.8	7.4	1.5	16
5.4	5.4	4.3	46				

Exp. 7. Leaves of *S. hastata*. Biological Station (Murmansk), June 1926. Duration of hydrolysis 44 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.0	2.6	0	0	5.8	5.5	2.5	71
3.4	2.8	0	0	6.2	6.0	2.5	71
3.8	3.2	0	0	6.6	6.5	2.5	71
4.2	3.7	0	0	7.0	6.9	3.5	100
4.6	4.6	0	0	7.4	7.3	2.5	71
5.0	4.8	0.5	14	7.8	7.6	2.0	57
5.4	5.2	1.5	43				

Exp. 7. Leaves of *Gossypium hirsutum* L. The Turkestan Cotton Breeding Station No. 182, June 1926. Duration of hydrolysis 44 hours.

p_H		Change in reducing sugar (Experiment-Control)		p_H		Change in reducing sugar (Experiment-Control)	
Buffer	At end	mg.	%	Buffer	At end	mg.	%
3.0	3.4	0.4	10	5.8	5.8	4.1	100
3.4	3.8	0.8	20	6.2	6.2	3.1	75
3.8	4.2	0.6	15	6.6	6.6	2.4	58
4.2	4.6	0.8	20	7.0	6.9	2.2	54
4.6	4.8	2.3	56	7.4	7.2	1.9	46
5.0	5.0	3.5	85	7.8	7.4	1.4	34
5.4	5.4	2.9	71				

The optimal p_H values for the action of the salicinase of *Populus* are in general less than those for *Salix*, the greatest action of *Populus* salicinases being found at p_H 5.8–6.4 and that of *Salix* enzymes at p_H 6.0–6.9. It is of interest that the enzyme of *S. hastata* has a minimum of action at p_H 4.6–4.8 and the enzymes of middle-Asiatic species of *Salix* have a distinct action even at p_H 3.0–3.6. The values of salicin hydrolysis for this boreal species of *Salix* are generally less than for the others.

At p_H 5.3 for *P. Bachofenii*, 5.7 for *P. nigra*, 5.4 for *S. babylonica*, 5.4 for *S. oxica*, and 5.4 for *Gossypium hirsutum* it is possible to see a decrease in the hydrolysis of salicin by the enzyme. This decrease coincides probably with an isoelectric point of protoplasmic proteins, because the precipitation of proteins at their isoelectric points may carry down the enzymes and thus inactivate

them. As the investigations of Pearsall and Priestley [1923] demonstrated, the most important proteins in tissues capable of growth have isoelectric points lying between p_H 3.0 and 6.0. The isoelectric points of potato-tuber tissues and of soya-bean root-tips were found by Robbins and Scott [1925] equal to p_H 6.4 and 6.2-6.44 respectively, for the mycelium of *Gibberella saubinetii* the position of the isoelectric point is at p_H 6.2, for *Fusarium lycopersici* at p_H 5.5 and for *Fusarium oxysporum* at p_H 4.9. Pearsall and Ewing [1924] give for the isoelectric point of yeasts p_H 3.1-3.3 and for *Nitella* p_H 4.6-4.7.

The relative amounts of enzyme in the leaf tissues of the plants investigated were determined by special experiments with hydrolysis of salicin at p_H values optimal for each plant species. The duration of experiment was 43 hours at 31°.

	p_H (optimal)	Reducing sugar in mg.
<i>P. Bachofenii</i>	5·8	11·5
<i>P. nigra</i> v. <i>pannonica</i>	5·4	4·0
<i>P. candicans</i>	6·4	6·5
<i>S. babylonica</i>	6·6	16·5
<i>S. oxica</i>	6·9	5·5
<i>S. sp.?</i>	6·0	8·5
<i>S. hastata</i>	6·9	4·0
<i>G. hirsutum</i>	5·8	3·5

Similar relations are also revealed by experiments on the determination of the velocity constants of salicin hydrolysis by the various leaf enzymes. These experiments give the following results (the conditions as in previous experiments).

t = time in hours.

x = reducing sugar experimentally obtained.

$$y = \text{reducing sugar calculated from the formula } k = \frac{1}{t} \cdot \ln \frac{a}{a-y}.$$

	<i>P. Bachofenii</i>							<i>P. nigra</i>					
<i>t</i>	0	12	24	36	48	End		0	12	24	36	48	End
<i>x</i>	0	1.75	3.75	6.5	8.5	15.7		0	1.0	1.6	2.0	2.4	15.7
<i>y</i>	0	2.3	4.2	5.9	7.3	15.7		0	0.8	1.5	2.2	2.9	15.7
	$k=0.00571$							$k=0.00187$					
	<i>P. candicans</i>							<i>S. babylonica</i>					
<i>t</i>	0	12	24	36	48	End		0	12	24	36	48	End
<i>x</i>	0	1.5	3.0	4.0	4.8	15.7		0	4.5	9.5	12.5	15.5	15.7
<i>y</i>	0	1.5	2.8	4.0	5.1	15.7		0	5.6	9.2	11.6	13.0	15.7
	$k=0.00358$							$k=0.01607$					
	<i>S. oxica</i>							<i>S. sp.?</i>					
<i>t</i>	0	12	24	36	48	End		0	12	24	36	48	End
<i>x</i>	0	1.3	2.5	3.2	4.3	15.7		0	2.0	4.0	6.0	7.0	15.7
<i>y</i>	0	1.2	2.4	3.4	4.4	15.7		0	2.2	4.0	5.6	7.0	15.7
	$k=0.00298$							$k=0.00535$					
	<i>S. hastata</i>												
<i>t</i>	0	12	24	36	48	End							
<i>x</i>	0	0.5	1.5	2.0	2.2	15.7							
<i>y</i>	0	0.6	1.2	1.8	2.4	15.7							
	$k=0.00150$												

The coincidence of values obtained experimentally with those calculated from a monomolecular formula is very satisfactory.

SUMMARY.

1. A definite optimal hydrogen ion concentration exists for the action of salicinas from various species of *Populus* and *Salix*.
2. The different plants contain various amounts of the enzyme.
3. The presence of salicinase in *Gossypium hirsutum* confirms the view that this enzyme is not limited to the species of the Salicaceae, which was first expressed by Krauch [1880] who found salicinase in some Cucurbitaceae.

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