SUMMARY

1. The enzymic degradation of ovolecithin and certain other phosphoglycerides in ethereal solution by snake-venom phospholipase *A* is stimulated by Ca^{2+} ions. The optimum Ca^{2+} ion concentration varies between 40 and 80 μM, when the lecithin concentration varies between 1·3 and 3·3 mM.

2. The phospholipase *A* activity of moccasin venom is inhibited by ethylenediaminetetraacetic acid and by Zn^{2+} and Cu^{2+} ions, but not by iodoacetate or *p*-chloromercuribenzoate.

3. All the natural and synthetic L-α-lecithins studied lost one ester group/molecule of substrate in the presence of the enzyme. Synthetic DL-α-lecithins lost only 0·5 mole of ester/molecule of substrate, whereas synthetic β-lecithins did not undergo any enzymic hydrolysis.

4. Egg phosphatidylethanolamine was degraded by phospholipase *A* when the ethereal solution was adjusted to pH 7·0, and there is some evidence for the breakdown of phosphatidylserine and ethanolamine plasmalogen.

5. Inositol phosphoglycerides were not attacked by moccasin-venom phospholipase *A*.

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Inhibition of Glycosidases by Aldonolactones of Corresponding Configuration

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There is no need to stress the potential value in many connexions of a class of selective inhibitors for the different glycosidases. Levvy (1952) discovered that mouse-liver β-glucuronidase is very powerfully inhibited by saccharo-1:4-lactone, and the presence of this lactone in traces in saccharate solutions explained the powerful inhibitory action of the latter on this enzyme (Karunairatnam & Levvy, 1949). Saccharo-1:4-lactone, as such, or in the form of a saccharate solution, has been shown to inhibit β-glucuronidase from every source that has been studied. Conchie (1954) found that β-gluco-

sidase from rumen micro-organisms was inhibited by the 1:4- and 1:5-lactones of gluconic acid, but not by saccharo-1:4-lactone. These findings have been confirmed with β-glucosidase from other sources (Jermyn, 1955; Robinson, 1956). Although efficient inhibitors, the gluconolactones are not as powerful as saccharo-1:4-lactone.

It was decided to extend these studies to other types of glycosidase, and at the same time examine the specificity of response to a range of lactones. This work also served another purpose in that rumen liquor was screened for enzymes hydrolysing

the glycosides of some of the commoner sugars. Since rumen liquor was deficient in some of these enzymes, they had to be prepared from other sources, and attention was ultimately turned to the common limpet (*Patella vulgata*), which displayed an astonishing range of glycosidase activity. Limpet preparations hydrolysed all the glycosides employed in the present work, and several others besides (Conchie, Levvy & Marsh, 1956).

EXPERIMENTAL

Materials

All melting points are corrected.

p-Nitrophenyl α-D-glucoside, prepared as described by Montgomery, Richtmyer & Hudson (1942), had the constants recorded by them: m.p. 216°, $[\alpha]_D^{17} + 215°$ in water (*c*, 1·0).

o-Nitrophenyl β-D-glucoside, prepared as described for the galactoside (Seidman & Link, 1950), had m.p. 152°, $[\alpha]_D^{16} - 107°$ in water (*c*, 1·2); Montgomery *et al.* (1942) give the same constants.

Phenyl β-D-glucoside, prepared as described by Montgomery *et al.* (1942), had m.p. 175°, $[\alpha]_D^{15} - 71°$ in water (*c*, 1·0); Fischer & von Mechel (1916) give m.p. 175–176°, $[\alpha]_D - 72°$.

Phenyl α-D-galactoside, prepared as described for the glucoside (Montgomery *et al.* 1942), had m.p. 145°, $[\alpha]_D^{16} + 215°$ in water (*c*, 1·4); Helferich & Appel (1932) give m.p. 146°, $[\alpha]_D + 217°$.

o-Nitrophenyl β-D-galactoside, prepared as described by Seidman & Link (1950), had m.p. 193°, $[\alpha]_D^{17} - 51°$ in water (*c*, 1·0); Seidman & Link (1950) give m.p. 193–194°, $[\alpha]_D - 52°$.

Phenyl α-D-mannoside, prepared as described by Helferich & Winkler (1933), had m.p. 131–132°, $[\alpha]_D^{17} + 113°$ in water (*c*, 1·0); Helferich & Winkler (1933) give m.p. 132–133°, $[\alpha]_D + 114°$.

Phenyl β-D-mannoside, prepared as described by Helferich & Winkler (1933), had m.p. 174–175°, $[\alpha]_D^{15} - 71°$ in water (*c*, 0·9); Helferich & Winkler (1933) give m.p. 175–177°, $[\alpha]_D - 72°$.

Phenyl β-D-xyloside, prepared as described by Montgomery *et al.* (1942), had m.p. 178–179°, $[\alpha]_D^{15} - 48°$ in water (*c*, 0·9); Montgomery *et al.* (1942) give m.p. 178–180°, $[\alpha]_D - 49°$.

Phenolphthalein β-D-glucuronide was prepared biosynthetically (Talalay, Fishman & Huggins, 1946).

Phenyl *N*-acetyl-α-D-glucosaminide: the tetra-*O*-acetyl derivative was prepared as described by Roseman & Dorfman (1951) and deacetylated with barium methoxide; m.p. 244–246°; Roseman & Dorfman give m.p. 246–247°.

Phenyl *N*-acetyl-β-D-glucosaminide: the tetra-*O*-acetyl derivative was prepared as described by Helferich & Iloff (1933), the fusion being continued for 5 hr., and deacetylated with barium methoxide; m.p. 248–249°; Roseman & Dorfman (1951) give m.p. 249–250°.

D-Glucono-1:5-lactone (British Drug Houses Ltd.) was twice recrystallized from ethanol; m.p. 153°, $[\alpha]_D^{18} + 63°$ in water (*c*, 1·8): Hedenburg (1915) gives the same constants.

D-Glucono-1:4-lactone, prepared as described by Hedenburg (1915) from calcium gluconate, had m.p. 135°,

$[\alpha]_D^{18} + 67°$ in water (*c*, 1·8): Hedenburg (1915) gives the same constants.

D-Mannono-1:4-lactone, prepared as described by Nelson & Cretcher (1930), had the constants recorded by them: m.p. 151–152°, $[\alpha]_D^{15} + 51°$ in water (*c*, 1·0).

D-Arabono-1:4-lactone (L. Light and Co. Ltd.) was twice recrystallized from acetone; m.p. 95–96°, $[\alpha]_D^{15} + 71°$ in water (*c*, 1·0); Jensen & Upson (1925) give m.p. 96°, $[\alpha]_D + 73°$.

Saccharo-3:6-lactone, prepared as described by Smith (1944), had m.p. 146°, $[\alpha]_D^{15} + 42°$ in water (*c*, 1·0); Smith (1944) gives m.p. 149°, $[\alpha]_D + 45°$.

D-Galacturonic acid (Eastman Kodak Co.) was twice recrystallized from 90% ethanol; m.p. 158–159°, $[\alpha]_D^{15} + 54°$ in water (*c*, 1·0); Link & Dickinson (1930) give m.p. 159–160°, $[\alpha]_D + 54°$.

Racemic acid (British Drug Houses Ltd.) was twice recrystallized from water to remove *meso*-tartaric acid; m.p. 203–204°; Bischoff & Walden (1889) give the same m.p.

The following commercial products were used without purification: D-, L- and *meso*-tartaric acids (British Drug Houses Ltd.); D-galactono-1:4-lactone, L-gulono-1:4-lactone, D-ribono-1:4-lactone and α-D-glucoheptono-1:4-lactone (L. Light and Co. Ltd.); D-glucurono-3:6-lactone (Corn Products Refining Co.).

The following compounds were gifts: L-galactono-1:4-lactone (Dr W. W. Reid), saccharo-1:4-lactone (Professor F. Smith), xylono-1:4-lactone (Dr H. S. Isbell), D-mannurono-3:6-lactone (Dr C. A. Marsh).

Saccharate solutions were prepared from potassium hydrogen saccarate (British Drug Houses Ltd.) and the solutions were boiled before use for 30 min. at acid pH to cause one-third conversion into saccharo-1:4-lactone (Levvy, 1952).

Mucic acid (British Drug Houses Ltd.) was thrice recrystallized from water; m.p. 219°: figures varying from 206° to 225° are given in the literature. Before use, solutions were boiled for 1 hr. to cause maximum lactonization (Levvy, 1952).

Enzyme preparations

From rumen liquor. Cheviot sheep with permanent rumen fistulae were kept on a diet of 500 g. of hay, supplemented with 450 g. of grass cubes at 7 a.m. and again at 4 p.m., and with free access to water and salt lick. Rumen liquor withdrawn at 9.30 a.m. was strained through nine layers of surgical gauze and centrifuged at 10 000 g for 20 min. The deposit was washed on the centrifuge with 1% (w/v) NaCl solution and suspended in water ($\frac{1}{5}$ vol. of rumen liquor). Micro-organisms were broken down by shaking with grade 12 Ballotini beads (Chance Bros. Ltd.) in the Mickle disintegrator (Mickle, 1948) for 45 min. The suspension was centrifuged at 10 000 g for 30 min. and the cell-free supernatant was brought to pH 5·2 and 20% saturation with ammonium sulphate. After 30 min. at 0°, the precipitate was centrifuged off at 1500 g for 15 min., and the supernatant brought to 80% saturation with ammonium sulphate. The precipitate that formed after 30 min. at 0° was centrifuged off at 10 000 g for 30 min. and dissolved in water. The final volume of the enzyme preparation was arranged to give a suitable liberation of aglycone in the enzyme assay (140–180 μg. of *o*-nitrophenol, 90–130 μg. of *p*-nitrophenol, 40–50 μg. of phenol), and was usually about 10% of the original volume of rumen liquor. Samples of rumen liquor are of course very variable. The enzyme blank

in all assays by the phenol method was reduced to negligible proportions by the ammonium sulphate fractionation.

From lucerne seed. Lucerne seed (De Puits, France) was suspended in water (60 ml./g. of seed) with a glass homogenizer. After centrifuging off insoluble material at 10000 g for 30 min., the preparation was brought to pH 5·2 and fractionated with ammonium sulphate as above. The final fraction was dissolved in 60 ml. of water/g. of seed.

From limpets. Freshly dissected limpet 'visceral humps' were homogenized in water (10 ml./g. of moist tissue), and after freezing and thawing were freed from insoluble material by centrifuging at 10000 g for 15 min. Fractionation with ammonium sulphate was done as described above, and the final fraction dissolved in 2·5 ml./g. of tissue. This stock solution was kept at − 20° and diluted as required, according to the enzyme to be studied. Dilutions of the stock solution were approximately as follows: α-glucosidase, 1:5; β-glucosidase, 1:40; α-galactosidase, 1:7; β-galactosidase, 1:20; α-mannosidase, 1:5; β-mannosidase, 1:10; α-*N*-acetyl-glucosaminidase, 1:4; β-*N*-acetyl-glucosaminidase, 1:5; β-xylosidase, 1:7; β-glucuronidase, 1:400.

From mouse liver. · Mouse-liver β-glucuronidase was prepared as described by Levvy (1952).

Enzyme assay

Liberation of o- and p-nitrophenol. Optimum hydrolysis of *o*-nitrophenyl β-glucoside by a rumen preparation in phosphate–citrate buffer was found to occur at pH 5·4 and a substrate concentration of 5 mM (Conchie, 1954). The incubation mixture contained 0·5 ml. of enzyme preparation in a final volume of 4 ml., and the colour of the free aglycone was developed by addition of glycine–Na₂CO₃ buffer. The same procedure was arbitrarily adopted for other *o*- or *p*-nitrophenyl glycosides and enzyme preparations. In some instances, however, the substrate concentration was reduced to 2·5 mM.

Liberation of phenol. Hydrolysis of phenyl glycosides was followed by the procedure of Kerr, Graham & Levvy (1948), with Ilford no. 608 red filter in the Spekker absorptiometer to measure free phenol. Incubation of enzyme with substrate was carried out for 1 hr. at 37° in 0·05 M citrate buffer. The pH was arbitrarily fixed at 5·2 and the substrate concentration at 2·5 or 5 mM. The incubation mixture contained 0·4 ml. of enzyme preparation in a final volume of 1 ml. Suitable corrections were made for interference by uronic acids (Karunairatnam & Levvy, 1949).

Liberation of phenolphthalein. The liberation of phenolphthalein from 0·625 mM phenolphthalein β-glucuronide by mouse-liver β-glucuronidase in 0·125 M acetate buffer, pH 5·2, was measured as described by Levvy (1952). There was 0·5 ml. of enzyme preparation in the incubation mixture, final volume 4 ml. For limpet β-glucuronidase the same conditions were employed, except that the pH was arbitrarily fixed at 4·5.

RESULTS

Table 1 shows the results obtained when all the lactones were screened for inhibitory power at a concentration of 10 mM against α- and β-glucosidase, galactosidase and mannosidase, as well as β-xylosidase and β-glucuronidase. To conserve pure saccharo-1:4-lactone, boiled saccharate solution was

used in these tests, and boiled mucate solution was employed in place of the unknown galactosaccharolactone. Galacturonic acid (unboiled) was used as such since it does not form a lactone. Preliminary experiments with rumen preparations stressed the need to work with cell-free extracts; otherwise, inconsistent results were obtained, no doubt owing to permeability and other factors.

Rumen liquor was found to lack α- and β-mannosidase activity. The former enzyme was therefore obtained from lucerne seed (Hill, 1934), and the opportunity was taken to examine the α-galactosidase also present in the preparation (Hill, 1934). Traces of β-mannosidase activity have been reported in baker's and brewer's yeast (Adams, Richtmyer & Hudson, 1943), but we were unable to confirm this observation with the yeast strains at our disposal. A β-mannosidase has been described in the snail *Helix laeda* Gould (Nagaoka, 1949), and the enzyme was therefore sought in the more readily available mollusc, *Patella vulgata*, with successful results. Since *P. vulgata* is rich in β-glucuronidase activity (Dodgson, Lewis & Spencer, 1953), this enzyme was included in these tests. Mouse-liver β-glucuronidase was examined too, in preference to the similar enzyme in rumen liquor (Karunairatnam & Levvy, 1951).

The limpet preparation was found to possess all the enzymes of present interest, and most of the lactones already found to be inhibitory were retested at 10 mM with this preparation. Also included in these experiments were limpet α- and β-*N*-acetyl-glucosaminidase. The results are shown in Table 2, with the exception of the figures for β-mannosidase and β-glucuronidase, these enzymes being more fully dealt with in Table 1.

Above 70 % inhibition of enzyme activity, quantitative comparison of different inhibitors is not possible because the curve relating percentage inhibition to inhibitor concentration is asymptotic. All those lactones which were powerful inhibitors of at least one enzyme were therefore retested at concentrations which gave marked, but not excessive, inhibition. Table 3 shows the comparative effects of the inhibitors at these concentrations on the different enzymes. Saccharate solution was now replaced by pure saccharo-1:4-lactone, and mucate was omitted.

One factor that is diminished in the experiments in Table 3 is the effect of trace impurities in the lactone solutions. Thus to take an extreme case, 0·025 % saccharo-1:4-lactone present as impurity in any other lactone will lead to nearly 50 % inhibition of β-glucuronidase by a 10 mM solution of the latter. There is no doubt that the presence of saccharate explains the feeble inhibitory action of gluconate on β-glucuronidase under conditions that permit lactonization (Karunairatnam & Levvy, 1949;

Table 1. *Percentage inhibition of glycosidases from various sources by sugar acid lactones in a final concentration of 10 mM*

Sources of enzymes: mixed rumen micro-organisms; lucerne seed; limpet visceral hump; mouse liver. Assay by phenol, nitrophenol and phenolphthalein methods. Results are expressed as percentages of controls.

Inhibition (%)

Lactone	p-Nitro-phenyl α-gluco-side (2.5 mM) Rumen	o-Nitro-phenyl β-gluco-side (5 mM) Rumen	Phenyl β-gluco-side (5 mM) Rumen	Phenyl α-galactoside (5 mM) Rumen	Phenyl α-galactoside (5 mM) Lucerne	o-Nitro-phenyl β-galacto-side (2.5 mM) Rumen	Phenyl α-manno-side (5 mM) Lucerne	Phenyl β-manno-side (2.5 mM) Limpet	Phenyl β-xylo-side (5 mM) Rumen	Phenolphthalein β-glucuronide (0.625 mM) Limpet	Phenolphthalein β-glucuronide (0.625 mM) Mouse liver
α-D-Glucoheptono-1:4-	0	2	5	2	-2	0	0	2	7	0	0
D-Glucono-1:5-	73	95	99	7	-1	0	30	2	27	0	0
D-Glucono-1:4-	75	93	97	0	-1	82	23	1	17	0	0
D-Galactono-1:4-	0	7	7	2	33	2	2	2	0	0	0
L-Galactono-1:4-	0	5	0	4	-1	1	2	92	0	0	0
D-Mannono-1:4-	1	4	10	4	-2	2	78	1	21	0	0
L-Gulono-1:4-	2	0	0	3	0	14	1	41	17	0	1
D-Arabono-1:4-	3	22	28	4	0	0	8	2	72	0	5
D-Xylono-1:4-	2	7	1	4	0	1	2	0	6	0	1
D-Ribono-1:4-	0	5	6	2	4	1	0	0	0	0	2
D-Glucurono-3:6-	1	6	0	6	0	1	2	2	0	0	1
D-Mannurono-3:6-	1	2	6	6	-2	-2	0	0	8	69*	56*
Saccharo-3:6-	0	16	8	1	-3	4	0	2	0	49†	40†
Saccharate (boiled for 30 min.)	0‡	0‡	6‡	0‡	-4‡	0‡	1‡	0‡	0‡	46§	44§
Mucate (boiled for 1 hr.)	0	10	5	0	0	0	2	1		12	14
D-Galacturonic acid											

* Final concn. of inhibitor 3 mM.
‡ Final concn. of inhibitor 5 mM (concn. limited by solubility).
† Final concn. of inhibitor 0.01 mM.
§ Final concn. of inhibitor 0.5 mM.

Table 2. *Percentage inhibition of limpet glycosidases by selected sugar acid lactones in a final concentration of 10 mM*. Results are expressed as percentages of controls.

Assay by phenol and nitrophenol methods.

Inhibition (%)

Lactone	p-Nitrophenyl α-glucoside (2.5 mM)	o-Nitrophenyl β-glucoside (5 mM)	Phenyl α-galactoside (5 mM)	o-Nitrophenyl β-galactoside (2.5 mM)	Phenyl α-mannoside (5 mM)	Phenyl β-xyloside (5 mM)	Phenyl N-acetyl-α-glucosaminide (3 mM)	Phenyl N-acetyl-β-glucosaminide (3 mM)
D-Glucono-1:5-	64	96	5	59	0	92	0	0
D-Glucono-1:4-	71	96	3	61	0	92	0	0
D-Galactono-1:4-	8	33	39	89	0	1	0	0
D-Mannono-1:4-	8	0	0	2	88	3	0	15
D-Arabono-1:4-	18	3	4	2	0	0	0	0
D-Xylono-1:4-	1	24	0	41	1	83		
Saccharate (boiled for 30 min.)	0	0	0	5	5	5		

Table 3. *Effects of selected sugar acid lactones in relatively low concentration on glycosidases from various sources*

For further details, see Table 1.

Substrate	Source of enzyme	Concn. (mM)	Inhibition (%)					
			D-Glucono-1:5-lactone (0·3 mM)	D-Glucono-1:4-lactone (0·3 mM)	D-Galactono-1:4-lactone (0·9 mM)	D-Mannono-1:4-lactone (0·9 mM)	D-Xylono-1:4-lactone (0·9 mM)	Saccharo-1:4-lactone (0·0025 mM)
p-Nitrophenyl α-glucoside	Limpet	2·5	18*	19†	0	0	0	0
o-Nitrophenyl β-glucoside	Rumen	5	59	59	0	0	6	0
	Limpet		64	65	3	0	3	0
Phenyl α-galactoside	Limpet	5	0	0	0	0	0	0
o-Nitrophenyl β-galactoside	Rumen	2·5	0	0	52	1	0	0
	Limpet		59	59	54	0	4	1
Phenyl α-mannoside	Limpet	5	0	0	0	40	0	0
Phenyl β-mannoside	Limpet	2·5	0	0	0	49	0	0
Phenyl β-xyloside	Rumen	5	12	12	0	0	35	0
	Limpet		65	65	-2	-2	48	-2
Phenolphthalein β-glucuronide	Limpet	0·625	0	1	1	1	0	43
	Mouse liver		0	0	0	0	0	34

* 0·9 mM lactone caused 33% inhibition.   † 0·9 mM lactone caused 32% inhibition.

Levvy, 1952; Mills, Paul & Smith, 1953); this effect disappears when pure gluconolactones are employed (Table 1; cf. Marsh, 1955). Errors from this source were observed with other enzymes in preliminary experiments with impure lactones.

When it comes to the effects of individual lactones on individual enzymes, it is convenient to consider Tables 1–3 collectively. The most important point that emerges is that, with one exception, all the glycosidases studied, whether α or β, were powerfully inhibited by the aldonolactones with the same arrangement of secondary hydroxyl groups. α-Galactosidase was the exception: in rumen preparations it was unaffected by 10 mM galactonolactone, whereas in lucerne and limpet preparations there was less than 50 % inhibition.

β-Glucuronidase from mouse liver or limpet was unaffected by any compound other than saccharo-1:4-lactone and boiled mucate solution. Inhibition by these was very much more powerful than anything observed with the other glycosidases and lactones. The relatively feeble action of saccharo-3:6-lactone on β-glucuronidase (Table 1) has been shown to result from intramolecular rearrangement to form the 1:4-lactone (Levvy, 1952). It is interesting to note that the inhibitory power of mucate was not diminished after repeated recrystallization of the impure commercial product: the figures in Table 1 are very similar to those obtained previously (Levvy, 1952).

With the α- and β-mannosidases and glucosidases from whatever source, inhibition by the lactones was fairly selective inasmuch as a pronounced effect was seen only with the lactone of the correct configuration. In this group of enzymes, however, some small, but significant, inhibitory effects were noted with arabonolactone (cf. Jermyn, 1955). The arrangement of the secondary hydroxyl groups in this lactone is identical with that at carbon atoms 3, 4 and 5 in a mannoside or glucoside. β-Glucosaminidase, where the same argument applies, was slightly inhibited by arabonolactone, but by no other lactone studied (Table 2). Both gluconolactones caused slight inhibition of α-mannosidase from lucerne seed, but not from the limpet, whereas limpet β-glucosidase was feebly inhibited by both galactono- and xylono-lactone.

The most difficult results to interpret are those for β-galactosidase and β-xylosidase. Limpet β-xylosidase appears to be identical with the β-glucosidase in the preparation: both enzyme activities were equally affected by the gluconolactones. The affinity of this unspecific enzyme for both substrate and inhibitor seems to be less for xylose than for glucose derivatives, since xylonolactone, a powerful inhibitor of the β-xylosidase activity, acted only feebly on the β-glucosidase activity, as already noted. In the rumen, however, β-xylosidase

activity, although partly a side-action of the β-glucosidase, appears to be mainly the property of a distinct enzyme, since the gluconolactones never caused more than small inhibition of the hydrolysis of the β-xyloside.

In the rumen, β-galactosidase appears to be an individual enzyme, since only galactonolactone caused any marked inhibition. In the limpet, however, the results for the hydrolysis of the β-galactoside present a very confused picture, for which it is difficult to find any simple and comprehensive explanation. Apart from powerful inhibition by galactonolactone, there was slight inhibition by xylonolactone and fairly strong inhibition by the gluconolactones. Inhibition by the gluconolactones could not be increased beyond a maximum of about 60 % by increasing the inhibitor concentration (cf. Tables 2 and 3). This finding would appear to rule out the possibility that this enzyme, like β-xylosidase, is identical with β-glucosidase in the limpet. It would also appear to exclude the possibility that limpet β-galactosidase activity is due to a single enzyme, highly specific for the substrate, but less specific towards inhibition by the lactones.

Action of the tartaric acids on β-glucuronidase

The opportunity was taken in the course of this work to examine the effects of the isomeric tartaric acids on limpet and mouse-liver β-glucuronidase. Karunairatnam & Levvy (1949) suggested that this point might be worthy of investigation, but only L-tartaric acid was available to them, and this had no action on the mouse-liver enzyme in a concentration of 15 mM. Wong & Rossiter (1951) found that, in a concentration of 10 mM, L-tartaric acid caused 15 % and meso-tartaric acid 60 % inhibition of rabbit-blood β-glucuronidase. In the same concentration, L-tartaric acid was claimed by Mills et al. (1953) to produce 85 % inhibition of an ox-spleen β-glucuronidase preparation.

In the present work, D- and L-tartaric acids, racemic acid and meso-tartaric acid were tested, in a final concentration of 10 mM, on β-glucuronidase of limpet and of mouse liver, with 0·625 mM phenolphthalein β-glucuronide as substrate. Only meso-tartaric acid had any inhibitory action on β-glucuronidase, producing a 45 % inhibition of the enzyme from limpet and a 23 % inhibition of the enzyme from mouse liver. Commercial racemic acid had some action, but this disappeared on purification. In case intermolecular rearrangement in a tartaric acid solution might lead to the production of an inhibitor for β-glucuronidase, the effect of boiling the solutions for 1 hr. at acid pH was examined, but this did not influence the results. The asymmetric carbon atoms in meso-tartaric acid, it should be noted, have the same configuration as carbon atoms 4 and 5 in glucuronic acid.

DISCUSSION

Earlier work with β-glucuronidase and β-glucosidase (Levvy, 1952; Conchie, 1954) suggested that any glycosidase should be powerfully inhibited by the aldonolactone with identical configuration of the secondary hydroxyl groups, irrespective of the size of the lactone ring. With the exception of α-galactosidase, this was true of the glycosidases studied in the present work.

Saccharo-1:4-lactone and boiled mucic acid solution were completely specific for β-glucuronidase amongst those glycosidases studied. These inhibitors and the substrate for the enzyme constitute, however, a special case in the present experiments, inasmuch as there is $-CO_2H$ instead of $-CH_2OH$ at carbon atom 6. It is noteworthy that inhibition by mucic acid is not due to an impurity: in the light of the results presented above, this raises the suspicion that, in spite of evidence to the contrary (Levvy & Marsh, 1952), β-glucuronidase may have some action on β-galacturonides. Preliminary results indicate that the newly discovered β-galacturonidase activity of limpet preparations (Conchie et al. 1956) is inhibited by boiled mucic acid solution.

Mannono-1:4-lactone was completely specific for α- and β-mannosidase. It should, however, be realized that the degree of specificity of an enzyme towards glycosides of different sugars may not always be paralleled by its response to the different aldonolactones. Some lack of specificity was noted in the inhibitory actions of the gluconolactones, as well as xylonolactone and galactonolactone, although the effects were pronounced (Table 3) only with the gluconolactones, and then only with the limpet-enzyme preparation. Some of the minor effects might, of course, disappear if the different enzymes were separated from each other. If, however, inhibition of a glycosidase by a lactone is any criterion of enzyme specificity, one must conclude that limpet β-glucosidase and β-xylosidase activity are due to one and the same enzyme, and that the β-galactosidase activity of the same preparation results from the actions of at least two distinct enzymes.

α-Galactosidase is not the only exception to glycosidase inhibition by the corresponding aldonolactone. Preliminary results for limpet α-glucuronidase and α-galacturonidase activity (Conchie et al. 1956) indicate absence of inhibition by saccharo-1:4-lactone and boiled mucic acid solution respectively. The failure of boiled mucic acid solution to inhibit α-galacturonidase activity was first noted by Dr W. W. Reid (private communication), using a pectinase preparation and α-di- and tri-galacturonic acids as substrates.

One possible application of the inhibition of glycosidases by lactones is illustrated by the work of

Dr G. N. Festenstein (private communication) in this laboratory, who found that the hydrolysis of cellobiose by extracts from mixed rumen micro-organisms was almost completely inhibited by glucono-1:4-lactone, whereas the hydrolysis of sucrose by a rumen holotrich Protozoa preparation (Oxford, 1955) was quite unaffected by the lactone. This confirms the belief that the protozoal invertase is a β-fructosidase and not an α-glucosidase.

Ezaki (1940) and Horikoshi (1942) employed gluconic and galactonic acids, as well as their lactones, as inhibitors, in an attempt to distinguish different types of β-glucosidase and β-galactosidase activity in Taka diastase and emulsin. It was not realized, however, that lactonization could account for the effects of the aldonic acids in enzyme assays done at a slightly acid pH. This factor may explain the apparent difference between the emulsin type of enzyme, assayed at pH 6 and unaffected by the aldonic acids, and the Taka diastase type, assayed at pH 5 and inhibited by the aldonic acids (cf. Levvy, 1952). This work has been extended to xylosidase by Morita (1953).

SUMMARY

1. With the exception of α-galactosidase, α- and β-glycosidases from rumen liquor, lucerne seed, limpet visceral hump and mouse liver were power-fully inhibited by aldonolactones of corresponding structure and configuration.

2. In many instances, the aldonolactones were completely specific in their actions, but in others some cross-effects were noted that could usually be attributed to a lack of complete specificity on the part of an enzyme.

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