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Inhibition of Glycosidases by Aldonolactones of Corresponding Configuration

4. INHIBITORS OF MANNOSIDASE AND GLUCOSIDASE*

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It has been shown that the inhibitory power of D-galactonolactone and of D-fuconolactone (6-deoxy-D-galactonolactone) towards the appropriate glycosidases is dependent upon the proportion of the uncharacterized (1→5)-lactone formed in the aqueous solution of the crystalline (1→4)-lactone (Levvy, McAllan & Hay, 1962; Levvy & McAllan, 1963). This was explained by the reversal of the configuration of the ring (and of the optical rotation), compared with the pyranosyl substrate, that occurs in the galactose series when one passes from the (1→5)-lactone to the (1→4)-lactone. This argument does not apply to the glucose or the mannose series. Nevertheless, the inhibitory power of crystalline D-mannono-(1→4)-lactone towards mammalian α-D-mannosidase is greatly enhanced when partial conversion into the (1→5)-lactone is effected in solution. The same difference in inhibitory power was encountered when the two lactones were tested against limpet α- and β-mannosidase.

Mannono-(1→5)-lactone has long been known as a crystalline compound, but the literature suggests that it is difficult to prepare and purify (Hedenburg, 1915; Isbell & Frush, 1933). In the course of this work, however, mannono-(1→5)-lactone was found to be a relatively stable aldonolactone. It could be readily isolated after the usual bromine oxidation of mannose, and repeatedly recrystallized without decomposition.

It became necessary to reinvestigate the comparative inhibitory effects of glucono-(1→4)- and -(1→5)-lactone on α -and β-glucosidase. In earlier work (Conchie, 1954; Conchie & Levvy, 1957) the two gluconolactones were found to be very similar to each other in their inhibitory power for both these glycosidases from rumen liquor and from the limpet.

EXPERIMENTAL

Enzyme preparations

Mammalian. Epididymides (16 g.) from 20 adult rats were homogenized in water, and acetic acid–NaOH buffer, pH 5·0, was added to give a final concentration of 0·1 M. After adjusting the volume to 5 ml./g. of epididymis, the suspension was incubated for 1 hr. at 37°. It was centrifuged at 1500*g* for 15 min. and the supernatant was fractionated with $(NH_4)_2SO_4$. The fraction separating (10 min. at 10000*g*) between 20 and 80% saturation was dissolved in 3 ml. of water/g. of tissue to give preparation *a*, and 2 ml. portions were stored at −20°. Under these conditions the α-mannosidase activity was retained indefinitely. For experiments with this enzyme, preparation *a* was diluted 1:5, giving preparation *b*, which retained its α-mannosidase activity for several weeks at 0°. Daily dilutions (1:20) were made from preparation *b* for α-mannosidase assay as described below. The α-mannosidase activity of preparation *a* was 18000 *p*-nitrophenol units/ml. (see below). The α-glucosidase activity of preparation *a* was 120 phenol units/ml. (see below), and it was diluted 1:2 before use.

Limpet. A limpet preparation (2·5 ml./g. of tissue), made by ammonium sulphate fractionation of an aqueous extract as described by Conchie & Levvy (1957), had an α-mannosidase activity of 8000 *p*-nitrophenol units/ml. and a β-mannosidase activity of 2000 phenol units/ml.: it was diluted 1:25 before assay in both cases. The β-glucosidase activity was 36000 *o*-nitrophenol units/ml. (preparation diluted 1:150 before use), and the α-glucosidase activity was 540 phenol units/ml. (preparation diluted 1:7·5 for assay).

Enzyme assay

α-Mannosidase. The incubation mixture contained 0·5 ml. of M-acetic acid–NaOH buffer, pH 5·0, 1·5 ml. of 16 mM-*p*-nitrophenyl α-mannoside and 0·5 ml. of the diluted enzyme preparation. The volume was made up to 4 ml. with water or inhibitor solution. After 1 hr. at 37°, 4 ml. of 0·4 M-glycine–NaOH buffer, pH 10·5, was added, the mixture centrifuged and the liberated nitrophenol measured on the Spekker photoelectric absorptiometer with Ilford no. 601 violet filters (peak transmission 430 mμ). The usual enzyme and substrate controls were made. Acidified or buffered inhibitor solutions were diluted before test so as not to affect the pH of enzyme assay. These conditions of assay, recommended by Conchie & Hay (1959) for mammalian α-mannosidase, were arbitrarily adopted for limpet α-mannosidase.

β-Mannosidase. The following conditions were arbitrarily adopted for limpet β-mannosidase. The incubation mixture contained 0·2 ml. of 0·25 M-citric acid–NaOH buffer, pH 5·0, 0·2 ml. of 12·5 mM-phenyl β-mannoside and 0·4 ml. of the diluted enzyme preparation. The volume was made up to 1 ml. with water or inhibitor solution. After 1 hr. at 37°, 2·5 ml. of freshly diluted Folin–Ciocalteu phenol reagent (1:5, v/v) was added. Protein was removed

* Part 3: Levvy, McAllan & Hay (1962).

by centrifuging at 1500*g* for 3 min. and 2·5 ml. of the supernatant was measured into 5 ml. of N-Na_2CO_3. After development for 20 min. at 38° the colour intensity was read with the Spekker photoelectric absorptiometer, by using Ilford no. 608 red filters (peak transmission 680 mμ). A control was done for the colour given by the reagents, as well as the usual enzyme and substrate controls.

α-Glucosidase. This assay was done as described for β-mannosidase, except that the substrate was phenyl α-glucoside in a final concentration of 5 mM.

β-Glucosidase. This assay was done as described for α-mannosidase, except that the substrate was *o*-nitrophenyl β-glucoside in a final concentration of 5 mM.

Inhibitors

Melting points are corrected.

Mannono-(1→5)-lactone. This compound was prepared from mannose by two methods of oxidation, and from mannono-(1→4)-lactone by two methods of interconversion through the free acid. Both methods of preparation from the (1→4)-lactone were unreliable and gave poor yields. Evaporations were done in a bath at 40°.

(*a*) Oxidation of mannose with bromine. This was carried out in the presence of barium benzoate as described by Nelson & Cretcher (1930) for preparing the (1→4)-lactone. After removal of water at low temperature, the crude product, which contained a mixture of the two lactones, was taken up in a large volume of 95% (v/v) ethanol and treated with charcoal. After 24 hr. at 25°, the (1→5)-lactone (2·5–3 g. from 18 g. of mannose) separated as heavy, highly refractive octahedrons. Subsequent recrystallizations were carried out in the same way; $[\alpha]_D^{20}+122°$ (*c* 0·3 in water). Because of rapid decomposition above 150° (cf. Hedenburg, 1915) this compound melts over the range 152–164°. Isbell & Frush (1933) give $[\alpha]_D+115°$; m.p. 158–160° (Equiv. wt. by titration with NaOH, 174. Calc. for $C_6H_{10}O_6$, 178).

The infrared spectrum (potassium bromide disk: Unicam SP. 200 Infra-Red Spectrophotometer) showed an absorption peak at 1740 cm.$^{-1}$, consistent with a (1→5)-lactone (Barker, Bourne, Pinkard & Whiffen, 1958). The aqueous solution had pH 5–6, and on titration with alkali showed ring-fission at pH 7·5.

(*b*) Oxidation of mannose by gaseous O_2 in presence of a Pt catalyst. A preliminary account of this general method for converting aldoses into (1→5)-lactones has been given by Conchie, Hay & Levvy (1963). Evaporation of the aqueous solution to dryness *in vacuo* and crystallization of the residue from 95% ethanol gave the (1→5)-lactone in 30% yield. In only one instance was a small quantity of the (1→4)-lactone obtained on working-up the mother liquor.

(*c*) Preparation from the (1→4)-lactone, according to Hedenburg (1915). Mannono-(1→4)-lactone was converted into barium mannonate, and Ba^{2+} ions were precipitated with H_2SO_4. The filtrate was taken to dryness and the residue was crystallized twice from 95% ethanol to give the (1→5)-lactone in 15% yield.

(*d*) Preparation from the (1→4)-lactone, with Amberlite IR-120 (H^+ form). The (1→4)-lactone (500 mg.) in aqueous solution (13 ml.) was converted into sodium mannonate by titration with NaOH, and the ion-exchange resin (1·5 g.) was added. After stirring for a few minutes, the resin was filtered off. The filtrate (pH 2) was heated to 100° for 10 min., cooled and taken to dryness. Two crystallizations of the residue from 95% ethanol gave the (1→5)-lactone in 15% yield.

Mannono-(1→4)-lactone. This was obtained after bromine oxidation of mannose by concentrating the mother liquors from the crystallization of the (1→5)-lactone. It separated in clusters of fine, light, white needles, and was recrystallized from 95% ethanol to constant low inhibitory power towards mammalian α-mannosidase: m.p. 152–153°; $[\alpha]_D^{20}+50°$ (*c* 0·5 in water). Isbell & Frush (1933) give m.p. 151–152°; $[\alpha]_D+51·5°$ (Equiv. wt. by titration with NaOH, 174. Calc. for $C_6H_{10}O_6$, 178).

The infrared spectrum showed an absorption peak at 1765 cm.$^{-1}$, consistent with a (1→4)-lactone (Barker *et al.* 1958). The aqueous solution had pH 5–6, and on titration with alkali showed ring-fission at pH 8·5.

The 'most inhibitory solution' from mannonic acid. This was analogous to the solutions of constant inhibitory power for β-D-galactosidase and β-D-fucosidase (Levvy *et al.* 1962; Levvy & McAllan, 1963). Sodium mannonate was prepared in solution from the (1→4)- or (1→5)-lactone. The solution was brought to pH 2 with HCl and heated at 100° for 10 min. It had $[\alpha]_D^{21}+56°$ (*c* 0·36 in water). As indicated in the preparation of mannono-(1→5)-lactone, Amberlite IR-120 (H^+ form) could be used instead of HCl in making this solution.

Glucono-(1→5)-lactone. This compound (British Drug Houses Ltd.) was recrystallized from 95% ethanol: m.p. 151–153°; $[\alpha]_D^{20}+67°$ (*c* 1·0 in water). Isbell & Frush (1933) give m.p. 150–152°; $[\alpha]_D+66°$ (Equiv. wt. by titration with NaOH, 178. Calc. for $C_6H_{10}O_6$, 178). The infrared spectrum showed an absorption peak at 1720 cm.$^{-1}$, consistent with a (1→5)-lactone. The aqueous solution had pH about 5, and on titration with alkali showed ring-fission at pH 7·0.

Glucono-(1→4)-lactone. This was prepared as described by Isbell & Frush (1933) from calcium gluconate. It was recrystallized from ethanol: m.p. 132–134°; $[\alpha]_D^{20}+68°$ (*c* 1·0 in water). Isbell & Frush (1933) give m.p. 133–135°; $[\alpha]_D+68°$ (Equiv. wt. by titration with NaOH, 178. Calc. for $C_6H_{10}O_6$, 178). Absorption in the infrared showed a single peak at 1770 cm.$^{-1}$, consistent with a (1→4)-lactone. The aqueous solution had pH about 5, and on titration with alkali was indistinguishable from the (1→5)-lactone, presumably because of interconversion (see Fig. 6), and showed ring-fission at pH 7·0.

RESULTS

Inhibition of mammalian α-mannosidase by the mannonolactones

The mammalian α-mannosidase preparation was used exclusively in studying the changes in inhibitory power of solutions of mannonic acid and its lactones. Fig. 1 shows the inhibitory power at varying concentration of the different entities towards this enzyme preparation. Pure mannono-(1→5)-lactone was 500 times as powerful as pure mannono-(1→4)-lactone, the concentrations for 50% inhibition being 0·080 and 40 mM respectively. Conchie & Hay (1959) gave a figure of 20 mM under comparable conditions for mannono-(1→4)-lactone,

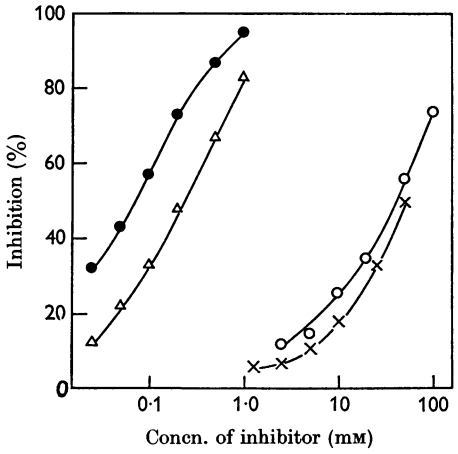

Fig. 1. Inhibition of mammalian α-mannosidase by varying concentrations of mannono-(1→5)-lactone (●), mannono-(1→4)-lactone (○), sodium mannonate (×) and the 'most inhibitory solution' derived from the mannonate ion (△) (see Experimental section). Inhibitor concentrations are on a logarithmic scale. In the control 90 μg. of *p*-nitrophenol was liberated.

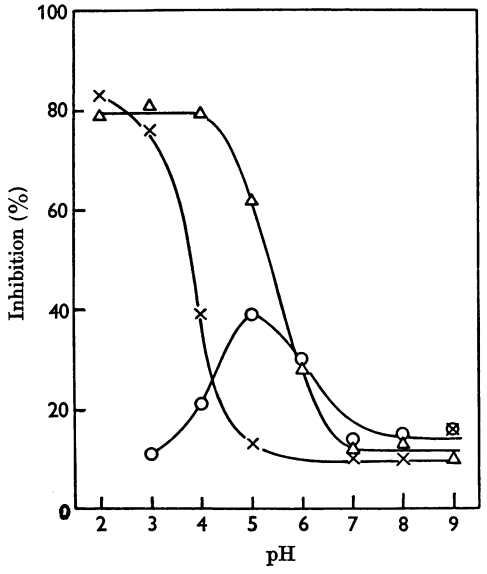

Fig. 2. Effect of heating at 100° for 10 min. in buffered solutions of varying pH on the inhibitory power of mannono-(1→4)-lactone (○), sodium mannonate (×) and the 'most inhibitory solution' derived from the mannonate ion (△) (see Experimental section). All tests were made at 1 mM concentration against rat-epididymal α-mannosidase. From pH 3 to 8 the buffer was 0·2 M-Na_2HPO_4–0·1 M-citric acid (McIlvaine, 1921). At pH 2, 0·1 M-citric acid was used, and at pH 9, 0·2 M-Na_2HPO_4. In the control 90 μg. of *p*-nitrophenol was liberated.

and this figure has been confirmed with their specimen. In general, different specimens of mannono-(1→4)-lactone with the same physical constants often varied in inhibitory power, until they were freed from last traces of the (1→5)-lactone by repeated crystallization. As shown in Fig. 1, the solution of maximum and reproducible inhibitory power that is obtained by heating a freshly acidified solution of sodium mannonate had an inhibitory power lying between the two lactones, with a 50 % value of 0·22 mM (in terms of mannonate ion). This corresponds to a mannono-(1→5)-lactone content of 36 % [ignoring the negligible contribution of the (1→4)-lactone]. From this figure and from the optical rotation (see Experimental section) it can be seen that only 25 % of the mannonic acid was in the form of the (1→4)-lactone. Mannonic acid causes virtually zero optical rotation (Hedenburg, 1915). The incomplete curve for sodium mannonate in Fig. 1 indicates that a solution added to the assay mixture, pH 5, had an inhibitory power only slightly lower than that of mannono-(1→4)-lactone, with a 50 % value of about 50 mM. [Since Ba^{2+} ions entered into two of the methods for preparing mannono-(1→5)-lactone, $BaCl_2$ was tested as an inhibitor. It had no effect at 10 mM.]

Both mannono-(1→4)- and -(1→5)-lactone acted competitively, with values for K_i, the inhibitor constant, of 32 and 0·071 mM respectively, compared with a value of 12 mM for K_m, the Michaelis constant, for *p*-nitrophenyl α-mannoside. Conchie & Hay (1959) give a value of 13 mM for this substrate and a rat-epididymal preparation.

Intramolecular transformations in mannonic acid solutions

After treatment of inhibitor solutions as illustrated in Figs. 2–5, they were tested against rat-epididymal α-mannosidase under the standard conditions of assay.

It can be seen from Fig. 2 that the (1→5)-lactone was rapidly formed from sodium mannonate in strongly acid solution at 100°. In other experiments, not illustrated, it was shown that at pH 2 and 100° maximum inhibitory power was attained after 7½ min., and remained unchanged up to 20 min., after which the inhibitory power fell by about 10 % over the next hour. There was little change in the specific rotation over the first 7½–20 min. Fig. 2 also shows the stability of the (1→5)-lactone in the 'most inhibitory solution', prepared as described in the Experimental section, on heating at 100° for 10 min. in buffers of varying pH. There was little change in inhibitory power up to pH 4. Above this pH, the inhibitory power fell, to approach the value for sodium mannonate at pH 7. Direct conversion of mannono-(1→4)- into mannono-(1→5)-

lactone was evident to a limited extent at pH 5 on heating at 100° in buffered solution for 10 min. (Fig. 2). Prolonged heating caused an overall fall in this pH–inhibition curve, without much change in shape.

The general proposition, that mannono-(1→4)-lactone can be transformed into the (1→5)-lactone without intermediate formation of sodium mannonate, is again illustrated in subsequent Figures. Fig. 3 shows that after 1 hr. at 38° and pH 7 there was formation of the (1→5)-lactone in a buffered solution of the (1→4)-lactone. At higher pH values, formation of the salt became evident, whereas at acid pH the (1→4)-lactone was stable. Fig. 3 also demonstrates the stability of the (1→5)-lactone, both in the form of the pure lactone and in the form of the 'most inhibitory solution'. The stability was greater in the latter case, and above pH 6 the inhibitory power fell, ultimately to coincide with the values for the (1→4)-lactone subjected to the same treatment.

The inhibitory power of a lactone inhibitor can be expected to fall as the pH of the solution approaches alkalinity. It can be seen from Figs. 2 and 3 that this applies to mannono-(1→5)-lactone, but that with the (1→4)-lactone there is first of all formation of a more inhibitory entity. Fig. 4 deals with the stability of the (1→4)-lactone over varying periods at 38° in buffered solution. At pH 4 the lactone was quite stable at 38°. At pH 6 there was formation of (1→5)-lactone, this process reaching a peak after 3 hr., and then a fall in inhibitory power, due, no doubt, to opening of the lactone ring. These changes were more rapid and pronounced at pH 7. Fig. 5 shows the stability of the pure (1→5)-lactone under similar conditions. At all pH values, there was a progressive fall in the inhibitory power of the solution, accompanied by a fall in optical rotation, indicating opening of the lactone ring, and perhaps (1→4)-lactone formation at pH 4: the higher the pH, the more rapid this process became. At 0°, the (1→5)-lactone was stable for at least 3 hr. at pH 4–6.

As may be expected from the relative stability of all these inhibitor solutions at pH 5, there was no

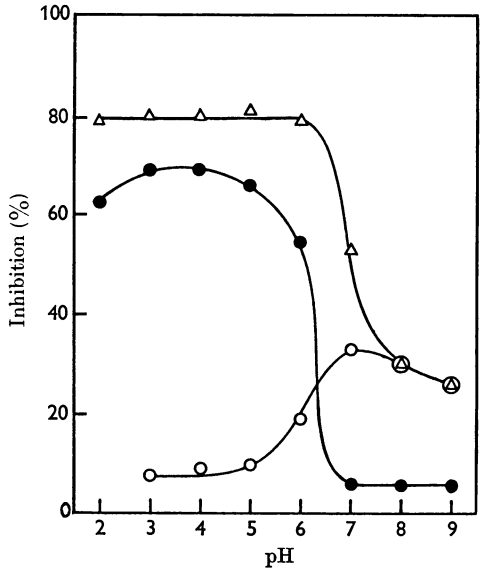

Fig. 3. Effect of incubation at 38° for 1 hr. in buffered solutions of varying pH on the inhibitory power of 1 mM-mannono-(1→4)-lactone (○), 0·25 mM-mannono-(1→5)-lactone (●) and the 'most inhibitory solution' derived from 1 mM-mannonate ion (△) (see Experimental section) towards rat-epididymal α-mannosidase. Buffers were as in Fig. 2. In the control 90 μg. of *p*-nitrophenol was liberated.

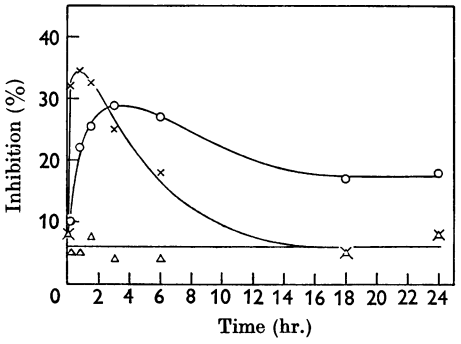

Fig. 4. Effect of incubation at 38° for varying periods on the inhibitory power of 1 mM-mannono-(1→4)-lactone in buffered solution at pH 4 (△), pH 6 (○) and pH 7 (×) towards rat-epididymal α-mannosidase. Buffers were 0·2 M-Na_2HPO_4–0·1 M-citric acid (McIlvaine, 1921). In the control 90 μg. of *p*-nitrophenol was liberated.

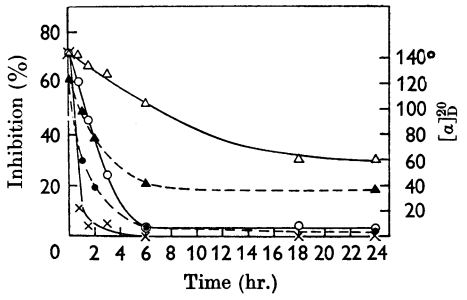

Fig. 5. Effect of incubation at 38° for varying periods: on the inhibitory power of 0·25 mM-mannono-(1→5)-lactone in buffered solution at pH 4 (△), pH 6 (○) and pH 7 (×) towards rat-epididymal α-mannosidase; and on the rotation of mannono-(1→5)-lactone (*c* 0·25) in buffered solution at pH 4 (▲) and pH 6 (●). Buffers were 0·2 M-Na_2HPO_4–0·1 M-citric acid (McIlvaine, 1921). In the enzyme control 90 μg. of *p*-nitrophenol was liberated.

detectable change with time in the percentage inhibition of mammalian α-mannosidase when it was incubated for periods of up to 2 hr. with substrate in the presence of mannonic acid or either of its two lactones, under the usual conditions of assay.

Inhibition of limpet α- and β-mannosidase by the mannonolactones

The concentrations of pure mannono-(1→4)- and -(1→5)-lactone required for 50% inhibition of limpet α-mannosidase were 26 and 0·098 mM respectively, and the corresponding values for limpet β-mannosidase were 7·1 and 0·013 mM.

Inhibition of mammalian α-mannosidase by other lactones

Tests were made of the inhibitory power of epimers of mannonolactone towards mammalian α-mannosidase. Neither D-talono-(1→4)-lactone (the C-4 epimer) nor L-gulono-(1→4)-lactone (the C-5 epimer) caused any inhibition whatsoever at 20 mM, whether tested in freshly prepared solution or after conversion into a 'most inhibitory solution' as described for mannono-(1→4)-lactone in the Experimental section.

Glucono-(1→4)- and -(1→5)-lactone (the two forms of the C-2 epimer) caused 50% inhibition of α-mannosidase at 23 and 8·2 mM respectively. This might suggest that the α-mannosidase and α-glucosidase activity of the preparation were due to a single enzyme. However, the α-glucosidase activity was very much lower than the α-mannosidase activity (see Experimental section), even allowing for the difference in aglycone in the two substrates, and mannono-(1→4)- and -(1→5)-lactone caused only 25 and 28% inhibition respectively of the α-glucosidase activity when both were tested at 20 mM. Moreover, the action of the gluconolactones on α-mannosidase was relatively feeble compared with their action on the α-glucosidase in the preparation (see below).

Inhibition of α- and β-glucosidase by the gluconolactones

Since glucono-(1→4)- and -(1→5)-lactone were found to differ slightly in their inhibitory power towards mammalian α-mannosidase, it was decided to reinvestigate their inhibitory effects on glucosidases. With the rat-epididymal preparation, the concentration for 50% inhibition of α-glucosidase was 1·3 mM-glucono-(1→4)-lactone and 0·85 mM-glucono-(1→5)-lactone: with limpet α-glucosidase the figures for 50% inhibition were 3·2 mM-glucono-(1→4)-lactone and 2·2 mM-glucono-(1→5)-lactone. The difference in inhibitory power, though small, was consistent in each case. A greater difference was observed in experiments with limpet β-glucosidase, which displayed 50% inhibition at 0·24 mM-glucono-(1→4)-lactone and 0·092 mM-glucono-(1→5)-lactone. Fig. 6 shows that at suitable pH it was possible to increase the inhibitory power of a solution of glucono-(1→4)-lactone towards limpet β-glucosidase, owing no doubt to partial conversion into the (1→5)-lactone. The effect was small, as one must expect from the small difference in the inhibitory power of the two lactones. Fig. 6 also illustrates the instability of glucono-(1→4)-lactone at a pH approaching neutrality, compared with both mannonolactones (cf. Figs. 4 and 5). Neither mannono-(1→4)- nor -(1→5)-lactone in a concentration of 20 mM produced any inhibition of this enzyme.

Since Fig. 6 indicates that glucono-(1→4)-lactone can increase in its inhibitory power towards limpet β-glucosidase on incubation at pH 5, the pH of enzyme assay, the possibility had to be envisaged that its action was dependent on rapid conversion into the (1→5)-lactone. From the velocity curves in Fig. 7, however, it can be seen that there was some increase in the inhibitory power of the (1→4)-lactone during the assay, but that this process was too slow to account for more than a small pro-

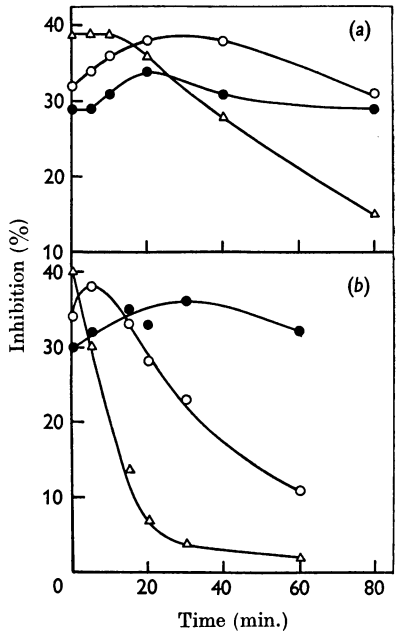

Fig. 6. Effect of maintenance at (*a*) 18° and (*b*) 38° on the inhibitory power of 0·1 mM-glucono-(1→4)-lactone in buffered solution at pH 5 (●), pH 6 (○) and pH 7 (△) towards limpet β-glucosidase. Buffers were 0·2 M-Na_2HPO_4–0·1 M-citric acid (McIlvaine, 1921). In the control 120 μg. of *o*-nitrophenol was liberated.

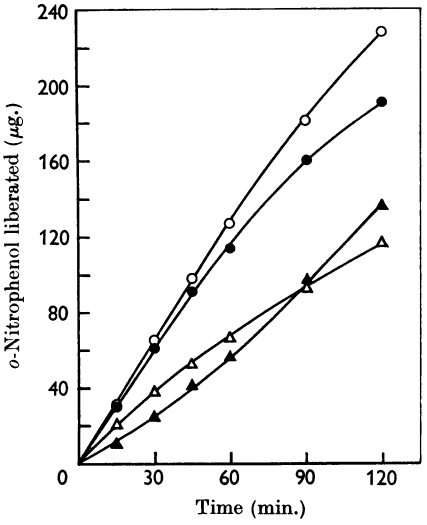

Fig. 7. Amount of *o*-nitrophenol liberated from 5 mM-*o*-nitrophenyl β-glucoside by limpet β-glucosidase on incubation for varying periods at 38° alone (○) and in the presence of: 1 mM-sodium gluconate (●), 0·3 mM-glucono-(1→4)-lactone (△) and 0·1 mM-glucono-(1→5)-lactone (▲).

portion of the inhibition observed. Fig. 7 also shows that, under the conditions of assay, glucono-(1→5)-lactone was not completely stable, and that lactonization of sodium gluconate was very slow.

DISCUSSION

The major question in this work arises from the great difference in inhibitory power between the two mannonolactones, since there is no obvious explanation on structural grounds such as applied to the analogous behaviour of the galactonolactones (see introduction). A possible explanation is that a free hydroxyl group at C-4 in the mannose residue is essential for combination with those mannosidases we have studied, or that the ring must be six-membered, as in the substrate. On the other hand, the gluconolactones differ only slightly in their inhibitory power towards glucosidases. Whereas there is quite a large difference in optical rotation between the mannonolactones, there is virtually none between the gluconolactones. A more general and more nebulous possibility, which would embrace galactonolactone, is that both the optical activity and the inhibitory power of a molecule depend on the same spatial considerations, when one compares the two forms of an aldonolactone. Conformational analysis of the sugars does not seem to cast any light at present on the difference between mannonolactone and gluconolactone as regards the effect of ring size (Reeves, 1951).

It is impossible to give a final answer to the second question, whether or not the very low inhibitory power of mannono-(1→4)-lactone is due to traces of the (1→5)-lactone present in the solid, or formed in aqueous solution. However, the inhibitory power of the (1→4)-lactone could not be completely abolished by repeated recrystallization, and it was relatively stable under the conditions of α-mannosidase assay, suggesting that it is a feeble inhibitor in its own right. In any case, under circumstances that permit interconversion of the two forms, the inhibitory power of a mannonolactone solution will be governed, for all practical purposes, by its (1→5)-lactone content. Changes in the inhibitory power of aqueous solutions provide information on the stability of the two lactones, and on their interconversion, that would be difficult, if not impossible, to obtain in any other way. Particularly noteworthy is the conversion of (1→4)- into (1→5)-lactone at neutrality, seen also with gluconolactone.

On the difference in inhibitory power between the two gluconolactones towards limpet β-glucosidase, it is impossible to say why this difference was not observed in earlier work with the same enzyme (Conchie & Levvy, 1957). There was no difference in physical constants, including the rates of mutarotation, between the specimens of the two lactones tested here and in earlier work. It is concluded from Fig. 7 that glucono-(1→4)-lactone is itself quite a powerful inhibitor of limpet β-glucosidase, compared with the (1→5)-lactone, a result which is consistent with the similarity between the two lactones in their inhibitory power towards α-glucosidase. There remains, therefore, a distinction between the gluconolactones on the one hand and the galactono- and mannono-lactones on the other hand, in the relative inhibitory power of the two lactone forms.

The final question, as to the identity or non-identity of α- or β-mannosidase and glucosidase in the preparations, was not studied, but on the evidence available from the present work for the mammalian preparation and from earlier work (Conchie & Levvy, 1957) for the limpet preparation they appear to be distinct enzymes in each case. It is remarkable that mannonolactone and gluconolactone each inhibit the corresponding glycosidase of both the α- and β-series, whereas other aldonolactones, for instance 2-acetamido-2-deoxygluconolactone (Findlay, Levvy & Marsh, 1958), have little, if any, action in the α-series.

SUMMARY

1. Methods for the isolation and purification of mannono-(1→5)-lactone are described. It was much more powerful than mannono-(1→4)-lactone as an inhibitor of mannosidases.

2. Mannono-(1→5)-lactone caused 50% inhibition of the hydrolysis of 6 mM-*p*-nitrophenyl α-mannoside at 0·080 mM by a rat-epididymal preparation and at 0·098 mM by a limpet preparation. The concentration for 50% inhibition of the hydrolysis of 2·5 mM-phenyl β-mannoside by the limpet preparation was 0·013 mM.

3. Concentrations of mannono-(1→4)-lactone causing 50% inhibition under the same conditions were 40 mM for rat-epididymal α-mannosidase, 26 mM for limpet α-mannosidase and 7·1 mM for limpet β-mannosidase.

4. Both mannonolactones acted competitively on rat-epididymal α-mannosidase, with values for K_i of 32 and 0·071 mM for the (1→4)- and the (1→5)-lactone respectively.

5. Glucono-(1→5)-lactone caused 50% inhibition of the hydrolysis of 6 mM-*p*-nitrophenyl α-mannoside at 8·2 mM by the rat-epididymal preparation, and of 5 mM-phenyl α-glucoside at 0·85 mM by the same preparation. With the limpet preparation the concentrations for 50% inhibition of the hydrolysis of 5 mM-*o*-nitrophenyl β-glucoside and 5 mM-phenyl α-glucoside were 0·092 and 2·2 mM respectively.

6. Concentrations of glucono-(1→4)-lactone causing 50% inhibition under the same conditions were 23 mM for rat-epididymal α-mannosidase, 1·3 mM for epididymal α-glucosidase, 0·24 mM for limpet β-glucosidase and 3·2 mM for limpet α-glucosidase.

7. Neither mannono-(1→4)- nor mannono-(1→5)-lactone caused any inhibition of the limpet β-glucosidase, and they were alike in their very feeble inhibitory power towards rat-epididymal α-glucosidase.

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Biochem. J. (1964) **91**, 384

Transport of Organic Compounds in the Mammal

PARTITION OF DIELDRIN AND TELODRIN BETWEEN THE CELLULAR COMPONENTS AND SOLUBLE PROTEINS OF BLOOD

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The investigations described in this paper are concerned with the problem of transport of two insecticides, dieldrin and Telodrin, in the blood of animals. We have measured particularly the distribution of these two substances between erythrocytes and plasma lipoproteins and proteins *in vivo* and *in vitro*. A knowledge of the transport of physiologically active substances which are not normally present in the body is fundamental to an understanding of the metabolism and the effect of these substances on animals.

MATERIALS AND METHODS

Insecticides. Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene) and Telodrin (1,3,4,5,6,7,8,8-octachloro-1,3,3a,4,7,7a-hexahydro-4,7-methano-isobenzofuran) were crystallized from acetone–methanol and acetone–hexane to constant melting point (dieldrin, m.p. 177°; Telodrin, m.p. 123°). The purified dieldrin and Telodrin migrated as single substances in reversed-phase paper chromatograms developed with two solvent systems (Mitchell, 1958); phenoxyethanol–$AgNO_3$ spray