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THE INHIBITORY ACTIVITIES OF 2-ACETAMIDO-2,3-DIDEOXY-D-HEX-2-ENONOLACTONES ON 2-ACETAMIDO-2-DEOXY-β-D-GLUCOSIDASE

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ABSTRACT

Treatment of 2-acetamido-2-deoxy-D-mannono-1,4-lactone with dicyclohexylamine in ethanolic solution afforded an unsaturated 1,4-lactone, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**1**), in good yield. 2-Acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,4-lactone (**2**) was similarly prepared from 2-acetamido-2-deoxy-D-galactono-1,4-lactone. An unsaturated 1,5-lactone, 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,5-lactone (**4**), was obtained through the oxidation of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-galactopyranose with silver carbonate on Celite, followed by mild hydrolysis.

The inhibitory activity of four isomeric 2-acetamido-2,3-dideoxy-D-hex-2-enonolactones [**1**, **2**, **4**, and 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**3**)] was assayed against 2-acetamido-2-deoxy-β-D-glucosidase from bull epididymis. Only the *erythro* lactones **1** and **3** are weak competitive inhibitors, whereas the *threo* lactones **2** and **4** are practically inactive. The 1,4-lactone **1** inhibited 2-acetamido-2-deoxy-β-D-glucosidase more strongly than the 1,5-lactone **3**. The lactones **1**–**4** were found to be quite stable in aqueous solution or under inhibitory-assay conditions.

In addition, two 2-acetamido-2-deoxy-D-glycals, 2-acetamido-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (**6**) and 2-acetamido-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol (**7**) were tested; both are 10 times as active as **1**.

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INTRODUCTION

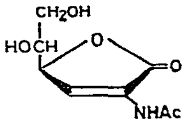
Present knowledge on the mechanism of the action of aldonolactones as enzyme inhibitors is still incomplete. The theory of action generally accepted is based on the conformational similarity between the lactone and the hypothetical, transition state in an enzyme-catalyzed hydrolysis[1]. A recent, comprehensive review dealing with this subject stated that the 1,5-lactones are generally[2] more powerful inhibitors than their corresponding 1,4-lactones. In addition, it has been suggested[3] that the inhibition is caused entirely by the 1,5-lactones, and that the apparent inhibition by a 1,4-lactone is a measure of its transformation into the former.

In our previous paper[4], the results of the examination of the inhibitory activity of pure samples of 2-acetamido-2-deoxy-D-gluconolactones and of their derivatives having fixed ring-size (isopropylidene acetals) towards 2-acetamido-2-deoxy-β-D-glucosidase (β-*N*-acetylglucosaminidase; 2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) were presented. It was found that the 1,5-lactone is undoubtedly more active, but the fact that 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone showed some activity suggested that the free 1,4-lactone was an inhibitor *per se*.

In the present paper, we report an extension of these studies to a new class of unsaturated lactones, the five- and six-membered 2-acetamido-2,3-dideoxy-D-hex-2-enonolactones. The results of the examination of the inhibitory activity of 2-acetamido-2-deoxy-D-glycals are also described.

RESULTS AND DISCUSSION

The synthesis of the lactones will be described first. The two unsaturated 1,4-lactones, namely, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**1**) and 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,4-lactone (**2**), had already been prepared[5]; when a 2-acetamido-2-deoxy-D-aldono-1,4-lactone (aldono = glucono, mannono, or galactono) was subjected to the action of methanolic potassium hydroxide, a mixture of the unsaturated lactones **1** and **2**, in approximately equal proportions, was formed; the process involved β-elimination, and epimerization at C-4. In the course of the present study, a modified method for the preparation of **1** and **2** was elaborated, based on the observation[6] that, under the action of anhydrous dicyclohexylamine, a saturated lactone slowly underwent a β-elimination. Treatment of



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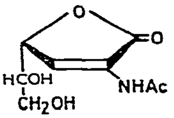

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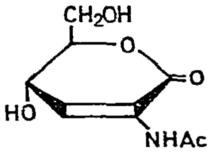
2-acetamido-2-deoxy-D-mannono-1,4-lactone[5,6] in anhydrous ethanol with dicyclohexylamine under carefully controlled conditions afforded pure lactone **1** in high yield. Lactone **2** was obtained in an analogous reaction from 2-acetamido-2-deoxy-D-galactono-1,4-lactone[5–7].

One of the pair of unsaturated 1,5-lactones, 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**3**) was recently synthesized[8]. Following the same procedure, 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,5-lactone (**4**) has now been prepared. Prolonged oxidation of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-galactopyranose[9] with silver carbonate on Celite led to the isolation of 2-acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-D-*threo*-hex-2-enono-1,5-lactone (**5**) which, on hydrolysis of the isopropylidene group, gave the lactone **4**.



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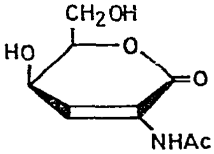

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5 4,6-*O*-isopropylidene

In passing, it may be noted that the free lactones **1**–**4** show optical rotations that are consistent with the lactone rule[10]. A certain regularity has been observed by comparing the optical rotations of the free lactones with those of their corresponding isopropylidene derivatives[5,8]. The data given in Fig. 1 show that the presence of an isopropylidene group in the *erythro*-lactones **1** and **3** causes change of the optical rotation from positive to negative values, whereas, with the *threo*-lactones **2** and **4**, the change is in the opposite direction. In all cases, the difference is almost constant (63–73°).

The inhibitory activity of the unsaturated 1,4-lactones **1** and **2** and the 1,5-lactones **3** and **4** was individually assayed in McIlvaine buffer (pH 4.8) against the 2-acetamido-2-deoxy-β-D-glucosidase from bull epididymis, using *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside as the substrate. The corresponding 5,6- and 4,6-*O*-isopropylidene lactone derivatives were tested in the same, standard manner[4].

The results obtained with the *erythro*-lactones **1** and **3** show that these unsaturated lactones may be regarded as competitive inhibitors toward the enzyme. However, the activity of the *threo* isomers **2** and **4** was practically negligible (see Fig. 2). This result stands in sharp contrast with the statement that 2-acetamido-2-deoxy-β-D-glucosidase is not specific for C-4, *i.e.*, that it hydrolyzes 2-acetamido-2-deoxy-β-D-glucosides and -galactosides[2,11], and is inhibited by 2-acetamido-2-deoxy-D-gluconolactones, as well as by 2-acetamido-2-deoxy-D-galactonolactone[12].

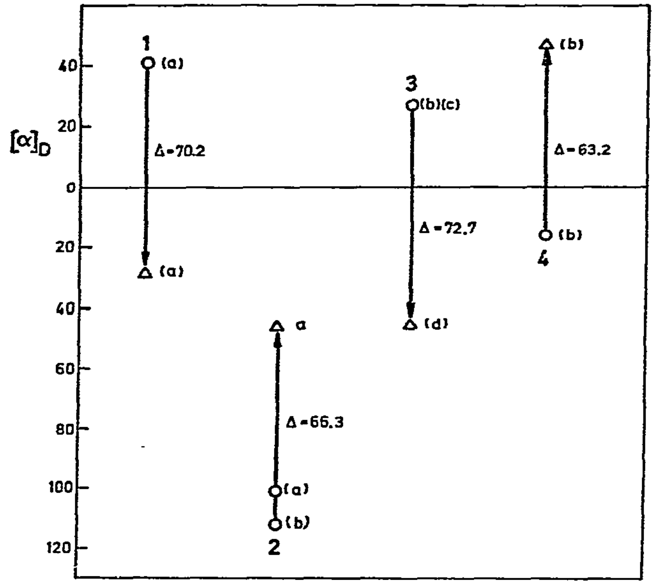


Fig. 1. Optical rotation of the free lactones 1–4 (in water) (○) and of their corresponding isopropylidene acetals (in chloroform) (△). [Data in (*a*) ref. 5; (*b*) this paper; (*c*) ref. 8, $[\alpha]_D$ +29.8° (in acetone); and (*d*) ref. 8.]

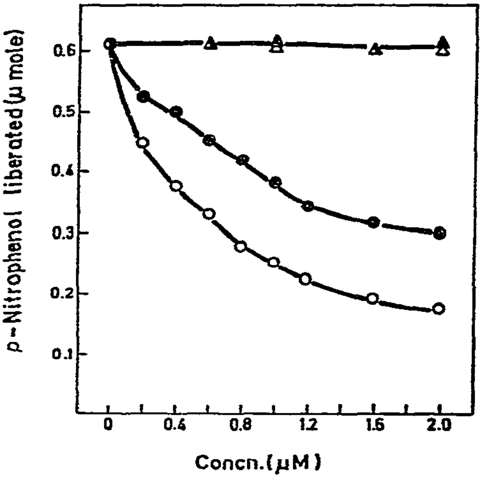


Fig. 2. Inhibitory activity of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (1) (○——○), 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,4-lactone (2) (△——△), 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (3) (●——●), and 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,5-lactone (4) (▲——▲) on 2-acetamido-2-deoxy-β-D-glucosidase from bull epididymis.

The 1,4-lactone **1** caused 50% inhibition at 0.6mM concentration, whereas the 1,5-lactone **3** required a concentration of 1.3mM (see Fig. 2). To our surprise, these values clearly show that the unsaturated lactones are weak inhibitors, about one thousandth as active as their corresponding, saturated lactones[4]. The isopropylidene acetals were found to have 0.25 to 0.33 of the activity of the free lactones **1** and **3** (see Table I). This is in full accordance with the earlier observation[4] that the presence of an acetal ring causes the decrease in activity.

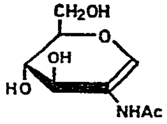
TABLE I

INHIBITION CONSTANTS (K_i) OF UNSATURATED AND SATURATED ALDONOLACTONES, OF THEIR ISOPROPYLIDENE DERIVATIVES, AND OF 2-ACETAMIDO-2-DEOXY-D-GLYCALS ON 2-ACETAMIDO-2-DEOXY-β-D-GLUCOSIDASE FROM BULL EPIDIDYMIS

Inhibitor	K_i
2-Acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone (**1**)	0.5mM
5,6-isopropylidene acetal	2.1mM
2-Acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,5-lactone (**3**)	1.5mM
4,6-isopropylidene acetal	4.8mM
2-Acetamido-2-deoxy-D-glucono-1,5-lactone	0.5μM
4,6-isopropylidene acetal	1.5μM
2-Acetamido-2-deoxy-D-glucono-1,4-lactone	4.4μM
5,6-isopropylidene acetal	1.5mM
2-Acetamido-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (**6**)	58μM[a]
2-Acetamido-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol (**7**)	40μM

[a] D. H. Leaback found the inhibition constant (K_i) to be 80μM when compound **6** was tested as an inhibitor of pig epididymal 2-acetamido-2-deoxy-β-D-glucosidase (personal communication).

As the unsaturated sugars are also effective as inhibitors of glycosidases[13–15], we extended our investigation to the examination of 2-acetamido-2-deoxy-D-glycals. 2-Acetamido-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol[16] (**6**) was found to be an efficient inhibitor of 2-acetamido-2-deoxy-β-D-glucosidase from bull epididymis; 2-acetamido-1,5-anhydro-2-deoxy-D-*lyxo*-hex-1-enitol[17] (**7**) was similarly active (K_i values are given in Table I), indicating that, here again, the enzyme does not show any specificity for C-4. The results obtained prove that 2-acetamido-2-deoxy-D-glycals are more potent inhibitors than the unsaturated lactones, the activity of lactone **1** being about one-tenth that of a glycal.



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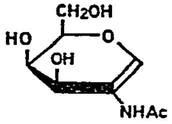

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In addition, the inhibitory activity of the 1,4-lactone **1** towards 2-acetamido-2-deoxy-*β*-D-glucosidases from different sources was determined; the results are summarized in Table II. In each case, the inhibitory action appeared to be competitive, but the activity varied with the origin of the enzyme.

TABLE II

INHIBITION OF 2-ACETAMIDO-2-DEOXY-*β*-D-GLUCOSIDASE FROM DIFFERENT SOURCES BY 2-ACETAMIDO-2,3-DIDEOXY-D-*erythro*-HEX-2-ENONO-1,4-LACTONE

Source	K_m[a] (*m*M)	K_i (*m*M)	*Concentration for 50% inhibition* (*m*M)	*pH*[b]
Bull epididymis	1.10	0.50	0.83	4.8
Horse liver	1.11	0.66	1.43	4.3
Rat liver	0.77	0.40	1.15	4.3
Almond emulsin	0.39	0.60	7.20	4.3
Pinto bean	0.46	0.88	8.3	4.6
Barley malt	0.33	3.06	>10.0	4.6
Limpet	3.81	6.25	>10.0	4.4
Aspergillus oryzae	0.85	2.10	3.8	4.5

[a]Substrate: *p*-nitrophenyl 2-acetamido-2-deoxy-*β*-D-glucoside; [b]McIlvaine buffer at optimal pH.

Under comparable conditions, the effect of the 1,4-lactone **1** on several glycosidases was tested. From the results, presented in Table III, it may be seen that only *β*-D-glucosidase was slightly inhibited at high concentration of the lactone. Thus, it is evident that the inhibitory action of the unsaturated lactone **1** on 2-acetamido-2-deoxy-*β*-D-glucosidase is highly stereospecific.

TABLE III

EFFECT OF 2-ACETAMIDO-2,3-DIDEOXY-D-*erythro*-HEX-2-ENONO-1,4-LACTONE ON SEVERAL GLYCOSIDASES

Enzyme (*source*)	*Substrate* (*m*M)[a]	*pH*	*Lactone* (*m*M)	*Inhibition* (%)
β-D-Glucosidase (almond emulsin)	3.3	4.8[b]	3.5	14
β-D-Galactosidase (bull epididymis)	5.0	3.1[b]	3.6	0
α-D-Mannosidase (bull epididymis)	6.2	4.5[c]	3.1	2
α-D-Mannosidase (almond emulsin)	6.2	4.5[c]	3.1	0
β-D-Glucuronidase (*Helix pomatia*)	3.0	4.5[c]	10.0	0

[a]Corresponding *p*-nitrophenyl glycosides; [b]McIlvaine buffer; [c]Sodium acetate buffer.

The most interesting aspect of our results, which appears to merit special comment, is the fact that the 1,4-lactones (free and protected) inhibit 2-acetamido-2-deoxy-β-D-glucosidase more strongly than the 1,5-lactones; for the free lactones, the activity differs by a factor of 3. The possibility that the free lactones **1** and **3** could be subject to interconversion and to formation of an equilibrium mixture should not be excluded. Therefore, the stability of these lactones in solution or under the conditions of the inhibition assays was carefully examined. The lactones **1**–**4** were found to be quite stable; the optical rotations for lactones **1**–**4** in water and for lactone **1** in McIlvaine buffer (pH 4.8) were examined over periods of 24 h, and mutarotation was not observed. After being incubated for 1 h under inhibitory assay conditions, lactone **1** was recovered unchanged in 80% yield (details in Experimental). Samples of the unsaturated lactones kept in aqueous solution or in McIlvaine buffer, even at room temperature, did not show any change in inhibitory activity during 30 days. The identity of a lactone, recovered from aliquots taken from the test solution at given intervals, was proved by characteristic absorptions in the infrared: (*a*) 1,4-lactones[5] have carbonyl absorption at 1750 cm^{-1}, 1,5-lactones at 1730 cm^{-1}; (*b*) *erythro*-lactone **1** shows[5] a unique band at 960 cm^{-1} which shifts to 915 cm^{-1} in the *threo* series (lactone **2**); lactone **3** could also be readily differentiated from lactone **4** by absorptions in the range 1000–800 cm^{-1}. The stability of these lactones should be compared with the behavior[4] of 2-acetamido-2-deoxy-D-glucono-1,4- and 1,5-lactones: activities of solutions of the saturated lactones increased or decreased with time, respectively, but both reached an equilibrium after 5 h.

These results confirming the unexpected stability of the unsaturated lactone systems, regardless of their ring size, called for further investigation. An inspection of the Dreiding models of the lactones **1** and **3**, and comparison with those of the corresponding saturated lactones, showed that the presence of the double bond might bring about an additional flattening of the ring, but it does not cause any essential change in the conformation of either the five- or the six-membered ring. It appears, then, that the stability of the unsaturated lactone systems might be ascribed to the electronic effects, which could be envisaged as the result of the conjugation of the double bond and the carbonyl bond, rather than to stereochemical factors.

In conclusion, it should be emphasized that, owing to the stability of the unsaturated lactone systems, our finding that the 1,4-lactone **1** is a more active inhibitor than the 1,5-lactone **3**, is even more significant. To the best of our knowledge, no such example has hitherto been described in the literature.

EXPERIMENTAL

General methods. — Specific rotations were measured at 20–24°. Column chromatography was performed on silica gel (E. Merck; particle size, 0.05–0.20 mm and 0.2–0.5 mm), with the following solvent-systems, all ratios being v/v: *A*, 13:4 dichloromethane–methanol; *B*, 1:1:2 ether–chloroform–acetone; and *C*, 1:1 chloroform–acetone. T.l.c. was conducted on silica gel in the solvent system specified.

Compounds were generally detected by charring with 10% sulfuric acid. Hydroxylamine–ferric chloride reagent was used to detect lactones specifically, and 0.5% potassium permanganate spray was used for the unsaturated lactones, which were also observable under u.v. light. I.r. spectra were recorded with a Perkin–Elmer spectrometer; n.m.r. spectra were recorded at 60 MHz with a Varian A-60*A* spectrometer, in the solvents specified, with tetramethylsilane as the internal standard. Optical absorptions were measured by means of a Beckman Acta V spectrophotometer.

The following materials were commercially available: frozen bull epididymis from Pel Freeze Biologicals Co.; β-D-glucosidase (almond), β-D-glucuronidase (*Helix pomatia*), *alpha*-amylase (*Aspergillus oryzae*), acetone powders (horse liver, rat liver, and limpet), and *p*-nitrophenyl glycosides from Sigma Chemical Co.; and diastase (barley malt) from Mann Research Labs.

*2-Acetamido-2,3-dideoxy-*D-erythro-*hex-2-enono-1,4-lactone* (**1**). — To a solution of 2-acetamido-2-deoxy-D-mannono-1,4-lactone (100 mg) in absolute ethanol (100 ml) was added dicyclohexylamine (0.15 ml) dropwise*, and the solution was kept for 10 days at room temperature, with the addition of a second portion (2 drops) of dicyclohexylamine after the second day. The solution was then concentrated *in vacuo* to 10 ml, diluted with pentane (3 ml), and refrigerated overnight. The crystals that deposited (73 mg, 78%) showed $[\alpha]_D$ +39.0° (water). After recrystallization from ethanol–pentane, pure **1** was obtained: m.p. 186–188°, $[\alpha]_D$ +40.0° (*c* 0.4, water). The infrared spectrum and chromatographic behavior were indistinguishable from those of an authentic sample[5]; the mixed m.p. was undepressed.

*2-Acetamido-2,3-dideoxy-*D-threo-*hex-2-enono-1,4-lactone* (**2**). — 2-Acetamido-2-deoxy-D-galactonolactone (400 mg) was treated as just described. After the solvent had been removed, the product was chromatographed on a column of silica gel (10 g) with solvent *A*. Fractions containing the unsaturated product (u.v.-positive spot) were evaporated to a syrup, which was dissolved in methanol and kept overnight in the refrigerator to yield crystalline lactone **2** (45 mg, 12%). After recrystallization from methanol, the compound had m.p. 176–178° and $[\alpha]_D$ −112.3° (*c* 0.4, water). The infrared spectrum was superposable on that of an authentic specimen[5]; the mixed m.p. was undepressed.

*2-Acetamido-2,3-dideoxy-4,6-*O*-isopropylidene-*D-threo-*hex-2-enono-1,5-lactone* (**5**). — To a solution of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-galactopyranose[9] (1.2 g) in anhydrous 1,4-dioxane (250 ml) was added dry silver carbonate on Celite (19.0 g). The mixture was mechanically stirred and boiled under reflux for 14 days; the progress of the reaction was monitored by t.l.c. in solvent *B*. The first products to appear were saturated lactones; the spot of the faster-moving unsaturated lactone **5** could be detected later, and, as the reaction progressed further, its intensity increased while the spots of the saturated lactones disappeared. At the

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*It is very important to use the exact amount of dicyclohexylamine, as otherwise, a by-product, dicyclohexylammonium 2-acetamido-2-deoxy-D-gluconate[5], could be formed, as has already been mentioned[6].

end of the reaction, the hot suspension was filtered through a layer of Celite, the precipitate was thoroughly washed with warm 1,4-dioxane, and the filtrate and washings were combined, and evaporated *in vacuo*; the residue was chromatographed on a column of silica gel (90 g) with solvent *B*.

Fractions that were chromatographically homogeneous and gave a positive test for unsaturation were pooled and evaporated, to give crystalline lactone **5** (240 mg, 20%) which was recrystallized from acetone–ether: m.p. 155–156°, $[\alpha]_D$ +47.3° (*c* 0.85, chloroform); +38.8° (*c* 0.67, acetone); ν_{max}^{KBr} 3440 (NH), 1720 (C=O), 1700 (C=C), and 1650 and 1520 cm^{-1} (Amides I and II); n.m.r. data (in $CDCl_3$): τ 2.01 (broad singlet, NH), 2.53 (doublet, $J_{3,4}$ 6.8 Hz, H-3), 5.43 (pair of doublets, $J_{3,4}$ 6.8 Hz, $J_{4,5}$ 2.4 Hz, H-4), 5.7–5.9 (multiplet, 3H), 7.88 (NAc), and 8.51 and 8.61 (CMe_2).

Anal. Calc. for $C_{11}H_{15}NO_5$: C, 54.76; H, 6.27; N, 5.81. Found: C, 55.02; H, 6.49; N, 6.01

*2-Acetamido-2,3-dideoxy-*D-threo-*hex-2-enono-1,5-lactone* (**4**). — To a solution of **5** (345 mg) in 2-methoxyethanol (20 ml) was added dry Dowex-50 X-8 (H$^+$) ion-exchange resin (3.5 g), and the mixture was kept at room temperature with occasional stirring for 3.5 h. The resin was then filtered off and washed with acetone, and the filtrate and washings were combined and evaporated to dryness *in vacuo*. The residue was chromatographed on a column of silica gel (30 g) with solvent *C*, 4-ml fractions of eluate being collected.

Fractions 13–16 contained unreacted lactone **5** (53 mg, 15%). From fractions 36–54 was obtained crystalline material (123 mg, 43%) which gave a positive test for unsaturation. It was dissolved in acetone; on scratching, it crystallized, affording pure **4**: m.p. 142–143°, $[\alpha]_D$ −15.9° (*c* 0.44, water), −24.2° (*c* 0.50, acetone); ν_{max}^{KBr} 3500–3300 (OH and NH), 1730 (C=O), 1670 (C=C), 1660 and 1540 (Amide I and II), 955, 945, 905, and 880 cm^{-1}; n.m.r. data (in acetone-d_6): τ 1.65 (broad singlet, NH), 2.43 (doublet, $J_{3,4}$ 6.4 Hz, H-3), and 7.88 (NAc). Prior to analysis, a sample was dried in high vacuum at 70°.

Anal. Calc. for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.72; H, 5.74; N, 7.15.

Enzyme preparation. — For enzyme preparations, commercially available materials were used, with the exception of six-day-old pinto-bean seedlings, which were cultivated in our laboratory.

Following the general procedure of Levvy and Conchie[18] and details given in our previous paper[4], the enzymes were extracted from freshly homogenized tissues or from acetone powders with 0.05M citric acid (pH 4–5) containing 0.1M sodium chloride; for some enzymes[18], acetate buffer was used. The stock preparation contained the fraction of extract that precipitated between 20 and 70% saturation with ammonium sulfate, and was dialyzed against the same buffer used for extraction.

Enzyme assay. — The incubation mixtures contained 1.0 ml of either McIlvaine buffer or acetate buffer (0.1M) at the optimal pH of the enzyme tested, the enzyme preparation (0.05 E.U.), and the corresponding *p*-nitrophenyl glycoside as the substrate. After dilution with water to a volume of 2.0 ml, the mixtures were incubated for 10–20 min at 38°, treated with 4 ml of 0.4M glycine–sodium hydroxide buffer (pH 10.4), and the absorbance measured at 430 nm. The inhibition constants, K_i, were obtained by plotting $1/v$ (μmole of *p*-nitrophenol liberated) *versus* I (mmole of inhibitor).

*Recovery of 2-acetamido-2,3-dideoxy-*D-erythro-*hex-2-enono-1,4-lactone* (**1**) *from incubation mixture.* — A mixture of McIlvaine buffer of pH 4.8 (10 ml), *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucoside (3.4 mg), the lactone **1** (35 mg), and 2-acetamido-2-deoxy-β-D-glucosidase from bull epididymis (0.5 E.U.) in water (20 ml) was incubated for 1 h at 38°, and then diluted with ethanol (5 ml) and filtered through a layer of carbon. The filtrate was concentrated *in vacuo* to give a semicrystalline residue, which was extracted with ether (3 x 5 ml) to remove *p*-nitrophenol, and finally with methanol (3 x 5 ml). The combined methanol extracts were evaporated and the residue was chromatographed on a column of silica gel with solvent *A*. The fractions containing unsaturated material (u.v.-positive spot) were pooled, and evaporated *in vacuo*, yielding crystalline 1,4-lactone **1** (28 mg, 80%). It was identified by analysis, m.p., optical rotation, and infrared spectrum.

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REFERENCES

1 D. H. Leaback, *Biochem. Biophys. Res. Commun.*, 32 (1968) 1025–1030.
2 G. A. Levvy and S. M. Snaith, *Advan. Enzymol.*, 36 (1972) 151–181.
3 T. E. Cooling and R. Goodey, *Biochem. J.*, 119 (1970) 303–306.
4 M. Pokorny, E. Zissis, H. G. Fletcher, Jr., and N. Pravdić, *Carbohydr. Res.*, 37 (1974) 321–329.
5 N. Pravdić and H. G. Fletcher, Jr., *Carbohydr. Res.*, 19 (1971) 339–352.
6 E. Zissis, H. W. Diehl, and H. G. Fletcher, Jr., *Carbohydr. Res.*, 28 (1973) 327–337.
7 P. Karrer and J. Mayer, *Helv. Chim. Acta*, 20 (1937) 407–417.
8 N. Pravdić, B. Danilov, and H. G. Fletcher, Jr., *Carbohydr. Res.*, 36 (1974) 167–180.
9 A. Hasegawa and H. G. Fletcher, Jr., *Carbohydr. Res.*, 29 (1973) 209–222.
10 C. S. Hudson, *J. Amer. Chem. Soc.*, 32 (1910) 338–346; 61 (1939) 1525–1528.
11 M. Pokorny and C. P. J. Glaudemans, *FEBS Lett.*, 50 (1975) 66–69.
12 J. Findlay, G. A. Levvy, and C. A. Marsh, *Biochem. J.*, 69 (1958) 467–476.
13 Y. C. Lee, *Biochem. Biophys. Res. Commun.*, 35 (1969) 161–167.
14 D. F. Wentworth and R. Wolfenden, *Biochemistry*, 13 (1974) 4715–4720.
15 E. T. Reese, F. W. Parrish, and M. Ettlinger, *Carbohydr. Res.*, 18 (1971) 381–388.
16 N. Pravdić and H. G. Fletcher, Jr., *J. Org. Chem.*, 32 (1967) 1806–1810.
17 N. Pravdić and H. G. Fletcher, Jr., *Croat. Chem. Acta*, 41 (1969) 125–133.
18 G. A. Levvy and J. Conchie, *Methods Enzymol.*, 8 (1966) 571–584.