Chapter 7
Hydrolysis of Glycosides

The glycosidic bond might be degraded by chemical and/or enzymatic agents. Comparative studies revealed that chemical hydrolysis is nonspecific and on the other hand the enzymatic is regiospecific and stereospecific. The glycosides are chemically susceptible to acid conditions and only in some cases to basic conditions. In general the acid sensitivity is attributed to the sugar moiety and the basic non-stability to the aglycon nature.

7.1 Acidic Hydrolysis

When a glycoside is subjected to acid conditions, a process called acetolysis takes place. This phenomenon is more clearly seen on *O*-glycosides where even weak acid conditions can be sufficient for *O*-glycoside breakage. Some simple glycosides such as β methyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranose are hydrolyzed under diluted HCl conditions to yield a hydroxy-2,3,4,6-tetra-*O*-methyl-D-glucopyranose. Likewise β ethyl-glucopyranose is hydrolyzed to a mixture of anomers (Scheme 7.1).

In general *S*-glycosides are more resistant than their counterparts *O*-glycosides to acidic medium; however, the former can be hydrolyzed under the conditions described in Scheme 7.2.

Disaccharides can be readily hydrolyzed under weak acidic conditions, producing their constitutive monomers in equivalent quantities (Table 7.1).

Depending on the strength of the hydrolytic conditions, polysaccharides undergo fragmentation, producing oligosaccharides, disaccharides, and monomers. The degradation degree relies on acid concentration, branching, and solubility. Thus, cellulose, being the most abundant natural polysaccharide in nature, requires high acid concentrations in order to be fully degraded to glucose. On the contrary some other polysaccharides at lower acid concentrations produce dimers and monomers (Table 7.2).

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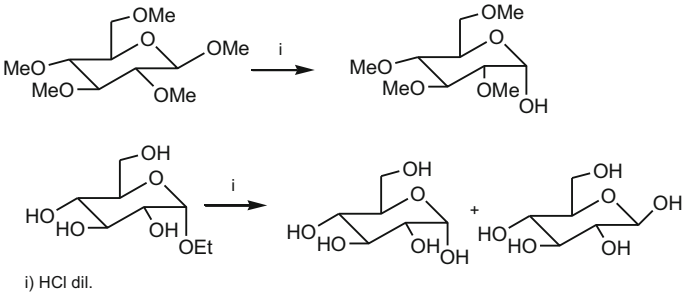


Scheme 7.1 Acid hydrolysis of simple *O*-glucosides

Scheme 7.2 Hydrolysis of thioglycosides

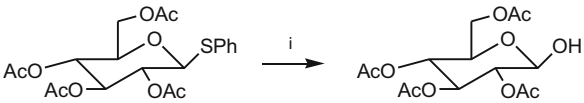


Table 7.1 Acid hydrolysis of disaccharides

Disaccharide	Hydrolysis product
(+)-Sucrose	D-(+)-glucose
	D-(−)-fructose
(+)-Lactose	D-(+)-glucose
	D-(+)-galactose
(+)-Cellobiose	D-(+)-glucose
	D-(+)-glucose

Table 7.2 Acid hydrolysis of polysaccharides

Polysaccharide	Partial hydrolysis	Total hydrolysis
Cellulose	1,4-cellobiose	D-glucose
Laminarin	1,3-laminaribiose	D-glucose
Curdlan	1,3-laminaribiose	D-glucose
Chitin	1,4-*N*-acetyl glucosamine	2-amino-2-deoxy-D-glucose
Mannan	1,4-mannobiose	D-glucose
Pullulan	1,4-maltotriose	D-glucose

Partial hydrolysis is important in certain cases in which disaccharides are not either affordable materials or easily obtained ones through synthetic means. Such is the case of 1,3-laminaribiose synthetically obtained in poor yields (9.5 %) [1], but readily available from polysaccharide curdlan [2].

Lewis acid hydrolysis of cellulose and methyl glycosides has been explored usually accompanied by heating. Thus, the conditions founded for achieving this goal were magnesium chloride in water with heating at 105 °C in either sealed or open vial [3].

7.2 Basic Hydrolysis

Some glycosides have been shown to be partially sensitive against basic conditions, besides their naturally high acid sensitivity. It is been experimentally founded that three classes of *O*-glycosides might be subject to basic hydrolysis [4].

(a) Phenolic glycosides
(b) Enolic glycosides
(c) β-substituted alcohol glycosides

7.2.1 Phenolic Glycosides

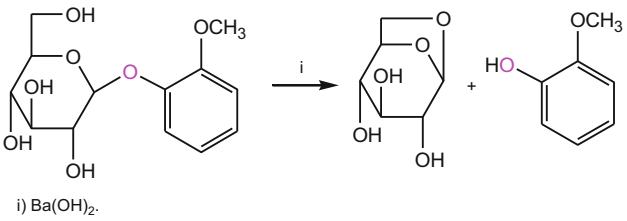
A typical example of phenolic glycoside decomposition under basic conditions is observed in the treatment of salicin with barium hydroxide giving as result a cyclic acetal and the release of the aglycon (Scheme 7.3).

7.2.2 Enolic Glycosides

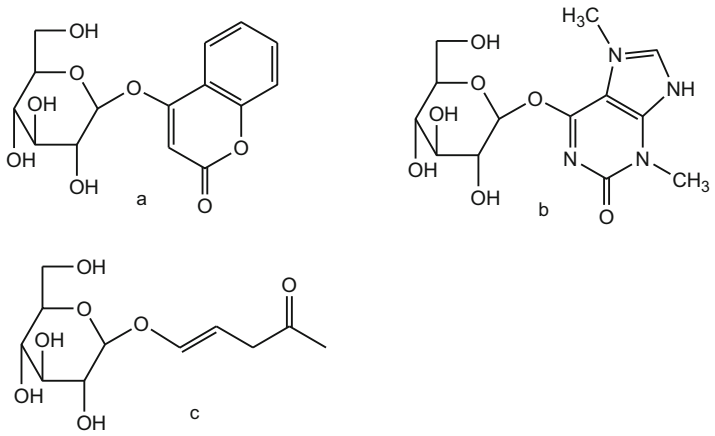
Within this type of glycosides, there are three varieties to be considered, which are: (a) 4-hydroxycoumarins, (b) purine and pyrimidine glycosides, and (c) simple enols (Scheme 7.4).

7.2.3 β-substituted Alcohol Glycosides

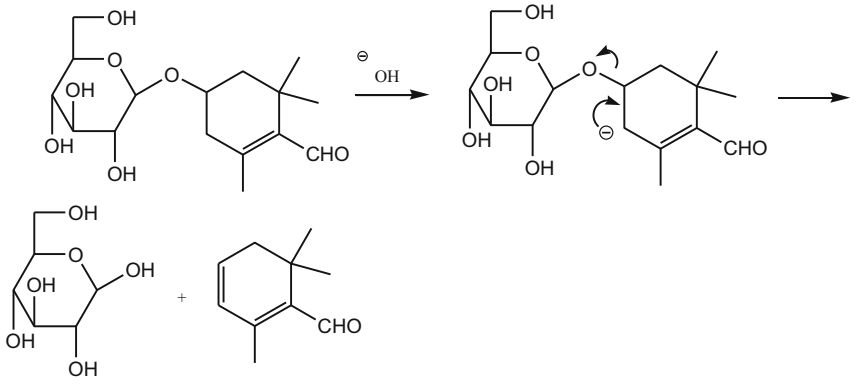
Glycoside picrocine is hydrolyzed in diluted potassium hydroxide solution, through a mechanism that involves a intermediate carbanion formation to give a conjugated unsaturated product and glucose as breakage product (Scheme 7.5).



Scheme 7.3 Basic hydrolysis of phenolic glycosides



Scheme 7.4 Basic hydrolysis of enolic glycosides



Scheme 7.5 Basic hydrolysis of β-substituted alcohol glycosides

Table 7.3 Degradation products of disaccharides under basic conditions

Disaccharide	Hydrolysis conditions (KOH) (N)	Temperature (°C)	Product
Cellobiose	1.5	50	Lactic acid
Gentobiose	2	50	Lactic acid
Lactose	0.2	100	D-galactose
Maltose	0.15	25	Phenylhydrazone of D-mannose

Contrary to acid hydrolysis of disaccharides where degradation products are their constitutive units, in most of the cases for basic conditions, non-sugar derivatives are produced (Table 7.3, Scheme 7.3).

7.3 Enzymatic Hydrolysis

β-glycosides are the natural substrates for hydrolytic enzymes known as β-glycosidases. So far, at biochemical level, the rule of most glycosidases is not totally well understood; however, some of them have been related to feeding, detoxification processes or even as a defense mechanism against herbivorous pathogens through release of thiocyanates, cyanides, and phytohormones. It has been established that there is a specific glycosidase for each aldopyranose, the sugar composition being responsible for the recognition pattern. Some of the best studied hydrolyses are the β-glycosidases and among them β-glucosidases, β-glucuronidases, β-glucanases, β-chitinases, all of them with important biological and economical implications [5].

7.3.1 β-glucosidases

There is strong evidence indicating that their action is mainly directed toward the defense mechanism and growth regulation. For instance cyanogenic glycosides are hydrolyzed, for the releasing of cyanide ions as a defense mechanism against animals. In humans the equivalent of β-glucosidase is called glucocerebrosidase (with low genomic homology to the plant counterpart) and catalyzes the degradation of glucosylceramide inside lysosome. The lack or deficiency of this enzyme produces the Gaucher disease characterized by accumulation of glucosylsphingosine and glucosylceramides.

7.3.2 β-glucanases, β-chitinases

The natural substrates for these oligosaccharide hydrolytic enzymes are laminarin and chitin, respectively, being present in fungi, yeast, and insects. Some of the processes related to the activity of these enzymes are: seed degradation, cellular elongation control, growth regulation, pollen growth regulation, digestion, and fertilization. Moreover, within the context of the defense mechanisms, these enzymes can be able to digest the fungi cellular wall, and also to release oligosaccharides that induce the production of antimycotic substances called phytoalexins.

7.3.3 β-cellulase

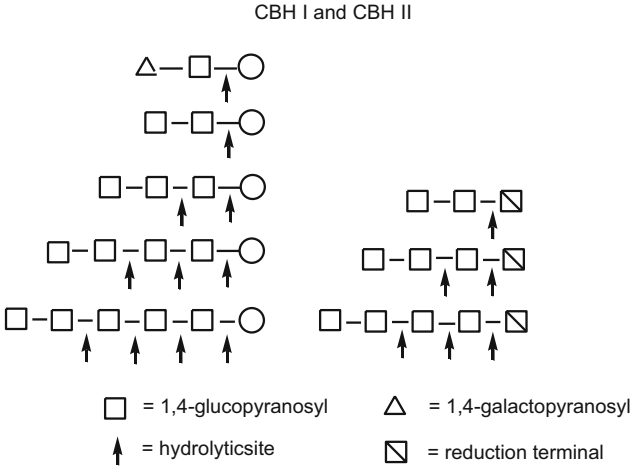
Cellulose is the most abundant natural polysaccharide on earth. Cellulytic enzymes particularly cellobiohydrolases CBHI, CBHII, EGI, and EGII found in fungi *Trichoderma reesei* have been thoroughly studied for determining the three-dimensional structure, the genomic sequence, receptors, and substrate specificity.

7.3.4 β-glucuronidase

In animals this enzyme is responsible for the detoxification processes, coupling mainly aromatic compounds and eliminating them as glucuronides. In plants there is not detectable β-glucuronidase activity; however, the development of the GUS gene fusion containing *E. coli* β-glucuronidase has been widely used as a gene marker [6]. Transgenic plants containing exogenic information fused to the β-glucuronidase gene marker can be conveniently monitored by using fluorogenic histochemical glucuronides.

7.3.5 Glycosidase Enzymatic Activity Detection

Detection can be achieved not only qualitatively, but also quantitatively, and for doing so high and low molecular weight substrates have been designed. Claeyssens [7] demonstrated hydrolytic specificity of cellulases CBH I and CBH II through the use of synthetic fluorogenic substrates containing the highly fluorescent coumarin umbelliferone or *p*-nitrophenol, in the form of *O*-glucosides. The cleavage of the glycoside releases the chromophore which can be easily measured in a fluorometer or spectrophotometer. The synthetic design of monosaccharides, disaccharides, trisaccharides, and tetrasaccharides attached to the mentioned chromophores has been of great advantage to determine the specificity during enzymatic cleavage (Scheme 7.6).



Scheme 7.6 Enzymatic specificity on low molecular weight substrates

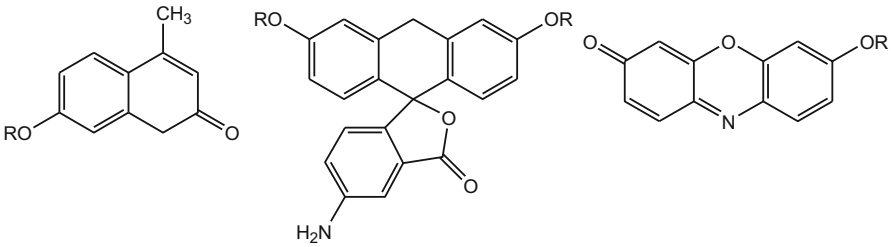
7.3.6 β-1,4-glucanases

The utilization of polysaccharides covalently attached to dyes has been reported. The complex Ostatin Brilliant Red-hydroxyethylcellulose (OBR-HEC)) is applied as a specific substrate for EG, Remazol Brilliant Blue-xylan (RBB-X)) the specific substrate for β-1,4-xylanases.

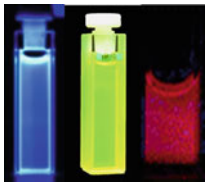
Likewise β-1,3-glucanases are detected by using an electrophoresis technique on polyacrylamide gels utilizing laminarin as substrate. The generated fragments are reacted further with azoic stain 2,3,5-triphenyltetrazolium to produce a color complex [8]. Despite their high sensitivity, this method cannot distinguish between endoglucanase and exoglucanase.

7.3.7 *Fluorescent* O-*Glycosides*

As mentioned before, fluorogenic aglycons are very useful molecules to monitor enzymatic activity. In principle, the fluorescent compound does not exhibit fluorescence in the glycoside form, and exerts its fluorescence when released as a result of the enzymatic activity (Scheme 7.7). Some of the fluorescent compounds widely used for enzymatic detections are: umbelliferone, fluorescein, and resorufin, having been coupled to most of the biologically important sugars as *O*-glycosides.

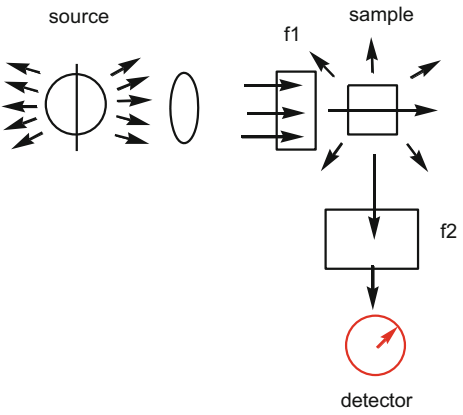


R = glucose, galactose, glucucronic acid, N-acetylglucosamine.

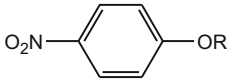


Scheme 7.7 Fluorescent *O*-glycosides and fluorescence emission after hydrolysis for (**a**) umbelliferone, (**b**) fluorescein, (**c**) resorufin

Scheme 7.8 Basic diagram of a fluorometer



Scheme 7.9 Absorption glycosides



The generated fluorescence is quantified in fluorometers constituted basically by a radiation source, and two monochromatic mirrors (f1 and f2). The first one selects the light for producing fluorescence activation, and the second transmits selectively fluorescence emission. A detector will measure the intensity of the fluorescence generated (Scheme 7.8).

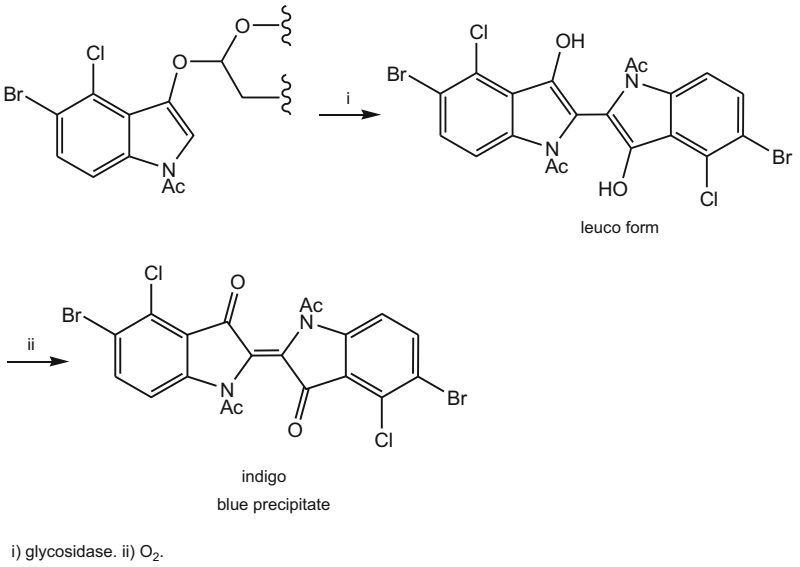
7.3.8 O-glycosides Measured by Absorption

Quantification of enzymatic activity following absorption detection is based in the use of synthetic *p*-nitrophenol in the form of *O*-glycosides as substrate (Scheme 7.9). The releasing of the aglycon from the sugar moiety produces slight yellow color measured as absorbance.

7.3.9 Histochemical O-Glycosides

Generally a histochemical substrate to be consider as a good candidate, should be such that in the form of *O*-glycosides it is water soluble and when the enzyme hydrolyzes the glycosidic bond releases the aglycon, which precipitates immediately. A compound that closely fulfills these requirements is 5-bromo-4-chloro-*N*-acetyl-3-indoxyl (X-gal, X-gluc, etc.) which has been attached to most of the biologically important monosaccharides, commonly identified as X-gal, X-gluc, etc. (Scheme 7.10).

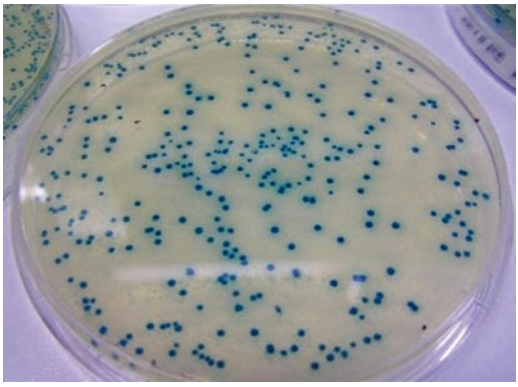
These chromophoric *O*-glycosides has been extensively used for detection of hydrolase activity and in molecular biology as screenable gene markers used to



i) glycosidase. ii) O_2.

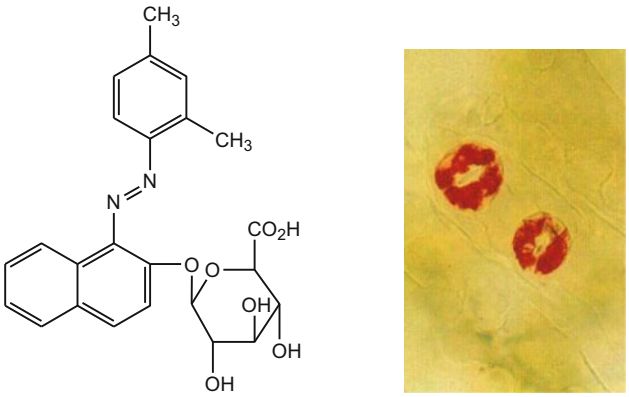
Scheme 7.10 5-bromo-4-chloro-indoxyl aldopyranose hydrolysis

Scheme 7.11 Genetically transformed bacteria cells containing lacZ gene expressing for β-galactosidase activity detected with X-Gal

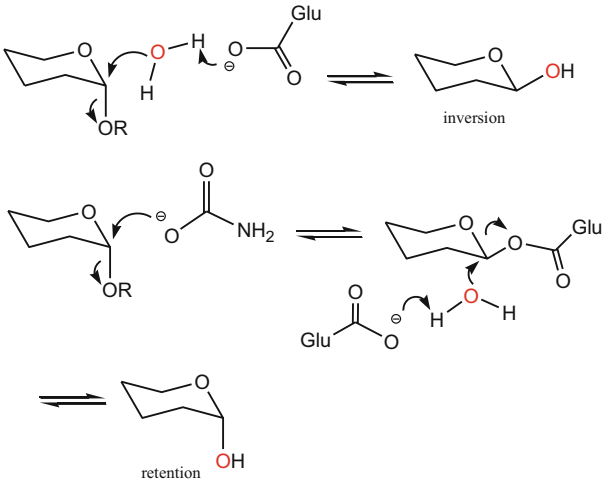


determine if a sequence has been successfully inserted in a cell known as the lacZ gene which encodes for β-galactosidase (Scheme 7.11). Although this is commercially available it is highly sensitive producing and easily detectable blue precipitate, it shows some diffusion before the monomers undergo dimerization in the presence of oxygen, to produce the blue indigo precipitate.

Alternatively, phenylazo naphthol *O*-glycosides (Scheme 7.12) known as Sudan glucuronides have been tested as a histochemical substrate for enzymatic detection of gene marker β-glucuronidase in transgenic plants [9, 10].



Scheme 7.12 Phenylazo naphthol glucuronides as histochemical substrates

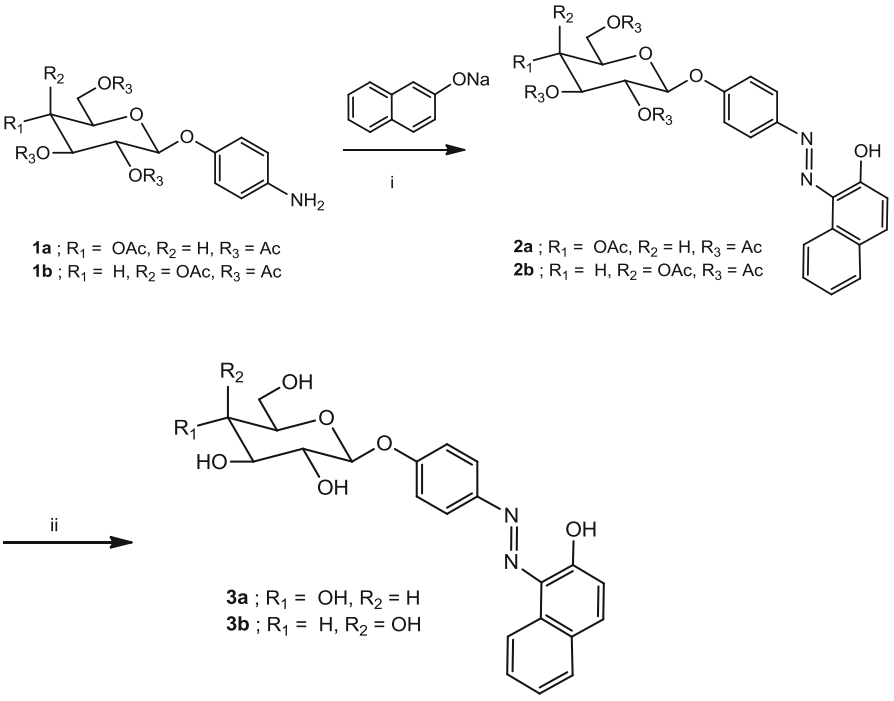


Scheme 7.13 Schematic representation of retention and inversion hydrolysis mechanism

The water-soluble Sudan glucuronide releases the phenylazo naphthol stain after enzymatic hydrolysis which can be seen in the sites of enzymatic activity as red crystals (Scheme 7.13). The mechanism and stereochemistry of enzymatic hydrolysis may occur with either inversion or retention of the configuration at the anomeric center. The first type of hydrolysis is carried out by the so-called inverting glycosidase, and the second by retaining glycosidase, with the vast majority of β-glucosidases being of the latter type. This has been proved through NMR studies, by measuring the chemical shift and magnitude of the coupling constant of the anomeric carbon. The most accepted mechanism involves protonation of substrate, participation of carboxylate attached to enzyme, glycoside–enzyme intermediate formation, and displacement as shown in Scheme 7.15 [12].

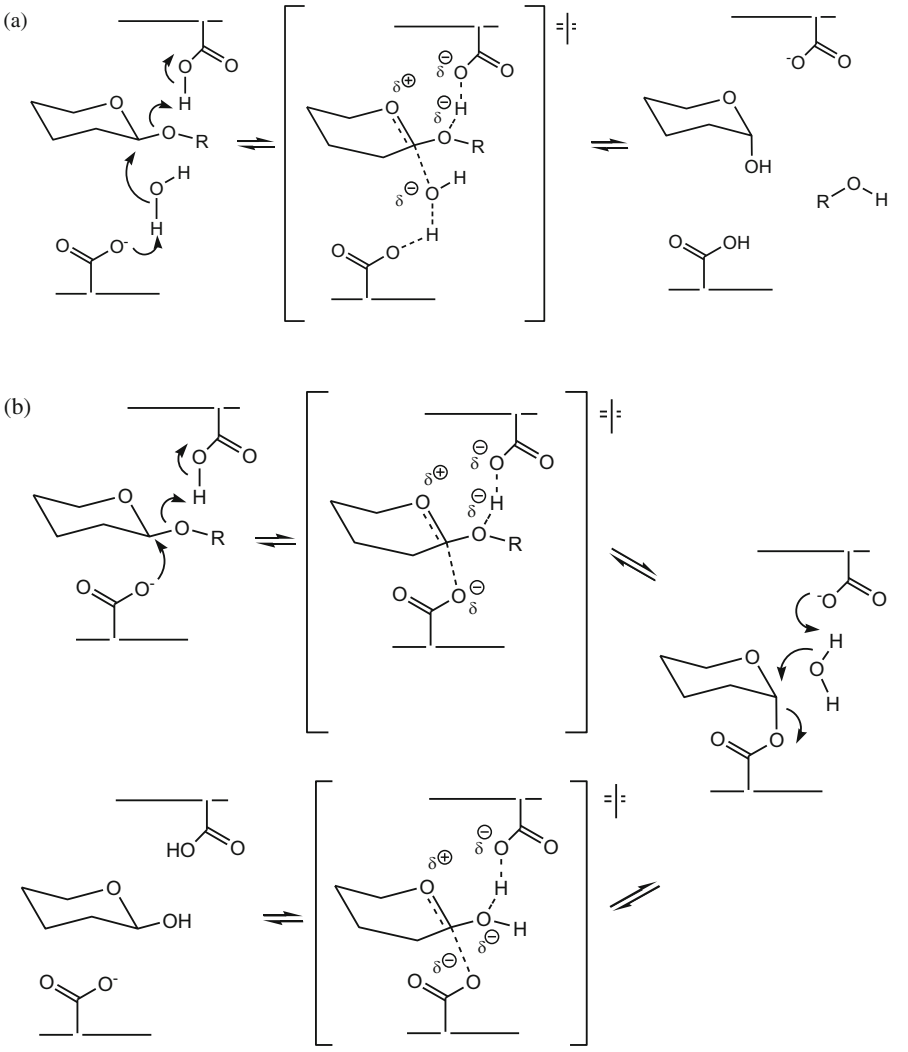
A suitable method for preparing azoic glycosides from aminophenyl glycoside precursor was performed under mild diazonium salt conditions, providing the corresponding protected azoic glycoside which after final deacetylation produce the azoic glycoside (Scheme 7.14) which was evaluated as substrates for detection of enzyme activity showing two maximum absorptions at 410 and 455 nm [11].

The partially water soluble Sudan glucuronide, releases the fenilazo naphthol stain after enzymatic hydrolysis which can be seen in the sites of enzymatic activity as red crystals. The mechanism and stereochemistry of enzymatic hydrolysis may occur with either inversion or retention of the configuration at the anomeric center. The first type of hydrolysis is carried out by the called inverting glycosidase, and the second by retaining glycosidase, being the vast majority of β-glucosidases of the later type. This has been proved through NMR studies, by measuring the chemical shift and magnitude of the coupling constant of the anomeric carbon. The most accepted mechanism involves, protonation of substrate, carboxylate participation attached to enzyme, intermediate formation glycoside-enzyme, and displacement as shown in Scheme 7.15 [12].



Conditions and reagents : (i) $NaNO_2$, AcOH, H_2O-THF, ^oC 30 min, then rt 1h. (iii) MeONa/MeOH, rt 30 min.

Scheme 7.14 General method for the preparation of azoic glycosides



Scheme 7.15 Schematic representation of inverting (**a**) and retaining (**b**) glycosidase mechanism

References

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