UDC 547

THE ALKALINE HYDROLYSIS OF FLAVONOID GLYCOSIDES

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Hydrolytic cleavage (by acids and enzymes) [1-3] is one of the methods of studying flavonoid glycosides.

Stepwise acid hydrolysis enables mainly 7- and 4'-glycosides to be obtained as intermediate products. 3-Glycosides are rarely obtained by this method, which makes difficult the investigation of complex glycosides.

In the literature available to us we have found no convenient method for obtaining 3-glycosides from flavonoid di- and polyglycosides.

In the course of a study of acylated flavonoid glycosides in which weak solutions of alkalis were used to split the esters, it was found that the hydrolysis of glycosides may take place under these conditions [4,5].

It is known that phenolic monoglycosides are capable of being hydrolyzed by alkalis, and the hydrolysis of flavonoid glycosides is usually accompanied by the decomposition of the aglycone, since in these cases, as a rule, fairly concentrated alkalis (5-25%) are used [6-8].

In our experiments, we attempted to carry out hydrolysis under milder conditions (0.2-2.0% solutions of KOH). Twenty different flavonoid glycosides were studied (table). Of a number of concentrations, a 0.5% aqueous solution of KOH proved to be the optimum for the hydrolysis of the flavonoid glycosides.

As can be seen from the table, the 7-monoglycosides of quercetin, kaempferol, and luteolin are cleaved completely in 10-30 min. Under these conditions the 3-monosides remain unchanged for more than 3 hr.

In the hydrolysis of diglycosides containing the sugars in positions 3, 7, and 4', the 3-glycosides are formed, and isosaponarin is cleaved to form saponaretin. Of the biosides, the 7- and 3-rhamnoglucosides differing from one another by the order of the bond between the sugars, were hydrolyzed. Under these conditions, as was to be expected, the 3-biosides and those of the 7-biosides in which the sugars were attached in the 1-2 position did not undergo hydrolysis.

According to Ballou et al. [6,9], the mechanism of the alkaline hydrolysis of the phenolic glycosides consists in the ionization of the 2-hydroxy group of the sugar in the alkaline medium, and this, in its turn, reacts with the glycosidic center, causing hydrolysis with the formation of a 1,2-epoxy derivative. The latter can then be converted into the 1,6-anhydro form. In this case, when the 2-hydroxy group is replaced by a second sugar, hydrolysis scarcely takes place [9]. We have observed this phenomenon in biosides with a 1,2-bond between the sugars (fortunellin, apinin, poncirin).

Alkaline Hydrolysis of Flavonoid Glycosides

Glycosides	Time of hydrolysis	Results
Quercetin 7-glucoside (quercimeritrin)	10 min	complete cleavage
Kaempferol 7-rhamnoside	10 min	complete cleavage
Luteolin 7-glucoside (cynaroside)	30 min	complete cleavage
Quercetin 3-glucoside (isoquercitrin)	3 hr	no change
Kaempferol 3-rhamnoside	3 hr	no change
Kaempferol 3-arabinoside	3 hr	no change
Quercetin 3-rutinoside (rubin)	3 hr	no change
Luteolin 7-rutinoside (scolimoside)	30 min	complete cleavage
Apigenin 7-rutinoside (isorhoifolin)	30 min	complete cleavage
Acacetin 7-neohesperidoside (fortunellin)	3 hr	no change
Isosakuranetin 7-neohesperiodoside (poncirin)	3 hr	no change
Isosakuranetin 7-acinobioside (acinoside)	30 min	complete cleavage
Apigenin 7-apioglucoside (apiin)	3 hr	no change
Kaempferol 3-arabinosido-7-rhamnoside	2 hr	kaempferol-3-arabinoside
Kaempferol 3,7-dirhamnoside	2 hr	kaempferol-3-rhamnoside
Isorhamnetin 3,4'-diglucoside (dactylin)	2 hr	isorhamnetin-3-glucoside
Kaempferol 7-rhamnosido-3-robinobioside (robinin)	2 hr	kaempferol-3-robinobioside
Isorhamnetin 3-glucosido-4'-rhamnoside (pasternoside)	30 min	isorhamnetin-3-glucoside
Apigenin 6-C-4'-O-diglucoside (isosaponarin)	30 min	apigenin-6-O-glucoside
Luteolin 8-C-glucosido-6'-O-xyloside (homoadonivernitol)	2 hr	no change

With respect to their behavior to alkalis, the glycosides can be classified as stable and labile. Alkali-sensitive are phenolic and enolic glycosides and glycosides of alcohols containing an acceptor group in the β position [6]. In accordance with this classification, the flavonoid glycosides can be distinguished as enolic (3-glycosides) and phenolic types. The glycosides of the second type, if they are not complicated by the structure of the carbohydrate substituents [9], are readily cleaved while the 3-glycosides proved fairly stable to the action of dilute alkalis.

This property is very important for the study of the diglycosides, since it enables the 3-glycosides to be separated from them, which facilitates investigation. In parallel stepwise acid hydrolysis it is possible to obtain glycosides with a free 3-hydroxy group. For example, by this method we have succeeded in establishing the structure of two diglycosides of the blackthorn (Prunus spinosa L.), which were characterized as kaempferol 3,7-dirhamnoside and kaempferol 3-arabinosido-7-rhamnoside.

In investigating C-glycosides, alkaline hydrolysis permits the O,C-diglycosides to be distinguished from O,C-biosides, which are not hydrolyzed under these conditions (isosaponarin, homoadonivernitol). Likewise, the alkaline hydrolysis of O,C-diglycosides does not form the isomeric products generally found in acid hydrolysis, which makes possible a faster and more accurate proof of the structure of this type of glycosides.

Experimental

The glycosides investigated (10-30 mg) were dissolved in 10 ml of 0.5% aqueous KOH and hydrolyzed in the boiling water bath for 3 hr. Samples for analysis were taken after 5, 10, 20, 30, 60, 120, and 180 min and were chromatographed on paper in several systems of solvents [15% acetic acid, butan-1-ol–acetic acid–water (4 : 1 : 5) etc.].

The intermediate glycosides were isolated by column chromatography on Kapron and were identified by their physicochemical properties, the products of acid hydrolysis, and spectral analysis in the UV and IR regions.

Conclusions

1. The alkaline hydrolysis of flavonoid glycosides has been studied.
2. In an alkaline medium 3-glycosides, C-glycosides, and biosides having 1,2 bonds between the sugars are stable.
3. The difference in the stabilities of the glycosides to alkalis can be used in studying flavonoid O-diglycosides, O-biosides with different positions of the bond, O,C-diglycosides, and O,C-biosides.

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