hyalospora. The epimeric hydroxylases (11α - and 11β -hydroxylases respectively) of both molds were stable to similar extents. Similarity of the cultures also appeared in the thermally unstable 11α -, 11β -, and 20β -HSD systems (see transformations of compounds I-IV).

Thus the various enzyme systems of hydroxylating microorganisms display different thermal stabilities. The unexpectedly high hydroxylase stability of cultures of *Th. orchidis* must be noted as must the marked change of direction of the process on thermal treatment of cultures of *C. blakesleeana*.

EXPERIMENTAL

Transforming cultures of Th. orchidis, Th. hyalospora, and C. blakesleeana were grown by the method in [1, 2]. Second-generation cultures of C. lunata grown by the method in [1] were used. Transformation by cultures of Th. orchidis and Th. hyalospora was carried out by washing 48-h mycelium from medium in 0.5% glucose solution, C. blakesleeana by washing mycelium from medium in tap water, and C. lunata by washing a 24-h mycelium from medium in pH 6.0 phosphate buffer. Experiments were carried out in 500-ml flasks with medium (50 ml). Cultures of Th. hyalospora, C. lunata, and C. blakesleeana in the medium for transformation were stirred at 40°C for 30 min, at 50°C for 20 min, and at 60°C for 10 min. Cultures of Th. orchidis were heated at 50°C for 30 min, at 60°C for 20 min, and at 70°C for 10 min, then substrates were introduced. Control experiments were carried out under standard conditions [1, 2]. The steroid for transformation (10 mg) was introduced in alcohol (0.9 ml). The culture mixture was extracted 3 times with equal volumes of chloroform. Products from the extracts were investigated chromatographically [2].

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CHEMICAL REACTION MECHANISM IN ACETONATION OF L-SORBOSE

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I. D. Chapanov, V. A. Nikiforov,V. V. Zarutskii, and A. A. Orekhov

The acetonation of L-sorbose (I) is one of the principal stages in the production of ascorbic acid.

Despite the low yield of the end product and the long duration of the reaction period in comparison with other processes in the production of ascorbic acid, the acetonation of I has not yet been systematically studied [1]. Most of the papers on the study of the acetonation describe the mechanism of chemical reactions.

It was found that acetonation catalyzed by sulfuric acid yields mainly 2,3:4,6-di-o-isopropylidene- α -L-sorbofuranose (II) [2]. Later, after a more thorough investigation of the reaction, monoacetone-L-sorbose was detected [3], which was identified as 1,2-o-isopropylidine- α -L-sorbopyranose (III). In acetonation in the presence of sulfuric acid, III was found in the solution in small amouts and, according to the literature data [3], could not be further acetonated.

Therefore, the initial mechanism was based on the concept of the formation at the first stage of two monoacetone-L-sorboses: 2,3-isopropylidene- α -L-sorbofuranose (IV) and III; the former is then converted into II, but III does not enter into further reaction [4].

The formation of the above components as the principal reaction products has been confirmed experimentally [5, 6].

Scientific Industrial Association Vitaminy, Moscow. Grozny Scientific-Industrial Association Promavtomatika. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 15, No. 10, pp. 76-79, October, 1981. Original article submitted October 24, 1980. The above mechanism has not been confirmed. The authors of [5] observed the reaction III \rightarrow II under conditions identical to those described in [3]. They also found that III is formed at the beginning of the acetonation, while IV appears at the later stage. These results disprove the initial acetonation mechanism.

A detailed study of the mechanism of the sulfuric acid acetonation was carried out by Tokuyama et al. [6]. For the investigation, the authors used pure compounds II, III, IV. By carrying out the acetonation of these compounds, they studied the kinetics of the following reactions: $I \rightarrow II$, $II \rightarrow IV$, and $IV \rightarrow II$. The authors found that the initial reaction product is component III, and gave the following mechanism for the acetonation reaction: $I \rightarrow III \rightarrow II \neq IV$. The reaction III \rightarrow II was considered to be a second order reaction, and it was suggested that an intermediate reaction $2III \rightarrow [III]_2$ takes place, in which two molecules of the pyranoside convert into dimer $[III]_2$, with subsequent transformation into II and formation of I.

Finally, the reaction mechanism had the following form [6]:

$$I \longrightarrow III \longrightarrow [III]_2 \longrightarrow II \rightleftharpoons IV.$$

A proton of the catalyst adds to III, forming a pyranoside ion III^+ , which then dimerizes into $[III]_2$. The protonated forms of III^+ and dimer $[III]_2$ were identified from changes in the UV spectrum of the solutions [6], but they were too active to be isolated in a pure state for further study.

Another hypothesis on the mechanism was proposed in [7, 8]. The authors detected compound III in a concentration of 8.4%, II in 28.3%, and IV in 11.2% of the theoretically possible amount 15 min from the beginning of the reaction, and therefore, suggested a dual structure for I: pyranoid (I) and furanoid (Ia). The following mechanism has been proposed for acetonation in two parallel paths:

$$I \longrightarrow III \longrightarrow II \rightleftharpoons IV,$$
$$Ia \longrightarrow IV \rightleftarrows II$$

The results obtained by the authors do not contradict the Tokuyama mechanism. In the studies of Tokuyama et al. [6], equilibrium in the reaction I $\stackrel{>}{\leftarrow}$ IV was established so quickly that when this reaction was carried out under strongly acid conditions, its rate could not be measured.

The presence of compunds II, III, and IV in the reaction mixture is not a sufficient proof of the hypothesis on the dual structure of I. It is possible that the authors of [8] observed the equilibrium in the reaction II \neq IV.

The Tokuyama mechanism is mainly confirmed by studies on the benzylidenation and ethylidentation of I [9, 10].

In acetonation, benzylidenation, and ethylidenation reactions, di- α -L-sorbofuranosides are formed from I via the intermediate stage of formation of α -L-sorbopyranose. In benzylidenation and ethylidenation processes, several other compounds are formed besides 1,2pyranosides and 2,3:4,6-di-o-furanosides.

To obtain products analogous to II without the formation of water causing hydrolysis of diacetonesorbose, we studied the process using dehydrated agents [11, 12], in particular, acetone diethylketal.

In the acetonation of I by acetone ketals, we also obtained several new mono- and diacetonesorboses [11, 12].

Similar compounds were also found in acetonation by acetone. First a new compound was detected, and then [5, 13] a large number of intermediate acetonation reaction components were identified. It was shown that if the acetonation is carried out in the presence of sulfuric acid, compounds II, III, and IV are mainly formed. Different results were obtained under weakly acid conditions, where the reaction rates are sufficiently slow to observe the intermediate components. The authors of [13] carried out the reaction in the presence of copper sulfate, and found that besides I, II, MII, and IV, 1,2-isopropylidene-sorbofuranose, 1,2: 4,6-di-o-isopropylidene- α -L-sorbofuranose, and several other compounds, which were later identified, are also formed.



Similar observations are described in [14, 15], whose authors studied the acetonation in detail. It was shown that the mechanism proposed by Tokuyama is for the overall process and does not describe a large number of intermediate stages and reaction components of the acetonation. However, in a strongly acid medium, the Tokuyama mechanism is a fairly good approximation, since all the components except II, III, and IV are unstable under these conditions and are not observed in the solution.

The new mechanism explains the results of all the previous investigations of the acetonation process, in general, including ethylidenation and benzylidenation [15]. It was shown that the transformation of the pyranoidal ring into the furanoidal one passes through the intermediate keto forms.

The authors of [15] presented the overall acetonation mechanism in the following form:

where V and V_k are 1,3-isopropylidene- α -L-sorbopyranose and its keto form, respectively; VI and VI_k are 1,3:4,6-di-o-isopropylidene- β -L-sorbofuranose and the keto form; VII and VIII are 1,3:4,6-di-o-isopropylidene- α - and β -sorbofuranoses.

Because of their instability during acetonation, compounds V and V_k were not detected, but in the benzylidenation and ethylidenation reactions of I, they were isolated.

Compounds VI, VII, and VIII are protonated in an acid medium to the keto form VI_k , which then converts into II as the most stable of the diketals. The intermediate compounds VI, VII, and VIII were observed in a weakly acid medium.

The mechanism of acetonation of I was studied mainly in systems with sulfuric acid and copper sulfate as the catalyst.

The acetonation on ion exchange resins proceeds on the inner and outer surfaces of the ion exchanger, and thus differs appreciably from systems studied in the literature.

The study of the acetonation mechanism of I on ion exchange resins has not yet been described in the literature, but it is probable that the mechanism of chemical reactions during catalysis by dissolved ions in the presence of sulfuric acid, and by counterions in the ion exchanger pores, is the same in character. This supposition is confirmed by the experimental data on other reactions, indicating that in catalysis by an electrolyte and an ion exchanger, the order of the reaction and the activation energy are the same [16].

Acetonation on the KU-23 cation exchanger was studied at the All-Union Scientific-Research Vitamin Institute, and revealed the presence in the solution of I, II, III, 2,3-monoacetone-sorbose, acetone, water, and mesityl oxide.

The presence of I, II, III, and IV as the main components in the reaction zone and the predominance of II over the remaining reagents makes it possible to suggest that the chemistry of acetonation on ion exchangers is similar to that of the reaction in a homogeneous medium [6].

It is clear that the KU-23 cation exchanger cleates strongly acidic conditions, under which all the intermediate compounds identified in the weakly acid media [13] are unstable and decompose before leaving the ion exchanger pores.

The mechanism of acetonation reactions was followed from experimental kinetic dependences. Figure 1 shows typical dependences of the concentrations of the principal components in the liquid phase on time, reflecting the kinetics of the chemical reactions taking place in the ion exchanger pores in a laboratory-type periodical mixing reactor.

Figure 1 shows that compound II predominates over the remaining components on the larger part of the kinetic curve.

The concentration of III first increases, passes through a maximum, and then slowly decreases, which is characteristic of the kinetics of the intermediate components [17]. With increase in the concentration of III, the rate of reaction III \rightarrow II increases until it becomes equal to the rate of reaction $I \rightarrow III$, and at this moment the concentration of III in the solution becomes stabilized. A slow decrease in the amount of III in the solution is due to a decrease in the amount of I consumed in the acetonation reaction.

In the kinetic studies, a marked dependence of the accumulation of II on the concentration of III is observed. This confirms the formation of II by the reaction III \rightarrow II.

In many experiments, compound IV appeared after the remaining components. This confirms the formation of IV by the reaction II $\stackrel{<}{\leftarrow}$ IV. Another confirmation is that kinetic curve 2 for IV qualitatively repeats curve 1.

Studies on the hydrolysis of II carried out in the absence of other components showed that IV is formed. Only in experimental studies carried out at high temperatures, traces of In experiments with pure IV, only II was obtained. III were detected.

The above confirms, in principle, the correctness of the mechanism proposed by Tokuyama [6] in the acetonation reactions on ion exchangers, which has the following general form:

 $I \longrightarrow III \longrightarrow II \rightleftharpoons IV.$

The authors of [6] observed a second order in the reaction III \rightarrow II, and therefore assumed the existence of an intermediate reaction $III \rightarrow [III]_2$ with the formation of complex [III]₂.

In the kinetic studies on the process on ion exchangers, reaction III \rightarrow IV was first order, and therefore we assumed that in this case, the system of equations of chemical reactions has the following form:

> $I + A \longrightarrow III + H_2O_2$ $III + A \longrightarrow II + H_2O_$, $II + H_2O \implies IV + A$.

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