were taken every 5 days, placed in counting bottles with ZhS-8, and incubated for 25 min, after which radioactivity was determined. The relative error of the determinations did not exceed 4%. It was found that the rate of evaporation of the pheromone from a rubber dispenser was not constant. In the first five days 60-65% of the substance deposited evaporated, and in the following days there was a slow uniform liberation of the pheromone with time. After 60 days, not more than 5% of the amount deposited remained. The results obtained agree well with practical results according to which dispensers retained their attractiveness for 45-50 days.

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USE OF EDIAP MASS SPECTROMETRY FOR DETERMINING COMPOSITION OF PRODUCTS OBTAINED IN PERIODATE OXIDATION OF SUCROSE

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The possibility has been demonstrated of using EDIAP mass spectrometry for the express determination of the composition of the products obtained in the periodate oxidation of sucrose and its subsequent reduction with $NaBH_4$. It has been shown that the method permits the amount of oxidized glycol and triol groupings to be determined and can therefore be used successfully to establish the structure of carbohydrates. The method also permits the recording directly in the reaction mixture of the presence of the equilibrium forms produced in the interaction of aldehyde groups with molecules of the solvent or of hydroxylamine. Analysis with the aid of EDIAP mass spectrometry requires 100-200 picomoles, calculated on the initial amount of sucrose. The time of analysis is 5 min. The mass spectra contain only the peaks of quasimolecular ions of the type of $[M + Na]^+$.

The analysis of a mixture of oligosaccharides is a fairly complex and laborious task which is solved by the successive use of various methods of fractionation and chemical modification, and the identification of the comopunds isolated with the aid of physicochemical methods [1]. It may be assumed that the use of a more universal mass-spectrometric method would permit the identification of such compounds without their fractionation and chemical modification which would considerably facilitate the solution of the problem.

Traditional methods of mass-spectrometric analysis and the determination of the structures of carbohydrates using electron-impact ionization or chemical ionization are used after their conversion into volatile forms: methyl or trimethylsilyl ethers or cyclic acetals [1]. The necessity for the preliminary modification of oligosaccharides and the considerable fragmentation during the ionization process limits the use of these methods and makes them unsuitable for the analysis of mixtures of these compounds.

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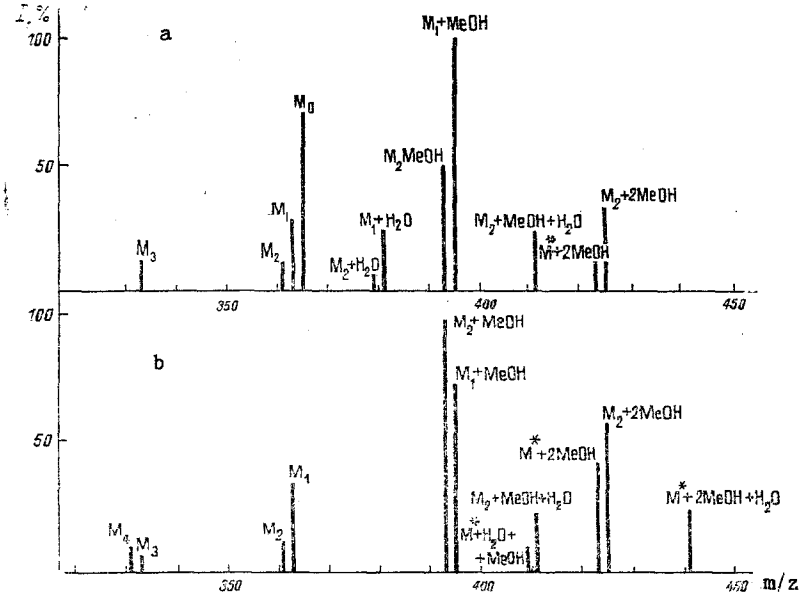

Fig. 1. Mass spectra of the reaction mixtures obtained in the oxidation of sucrose by sodium periodate at ratios of 1:1 (a) and 1:3 (b).

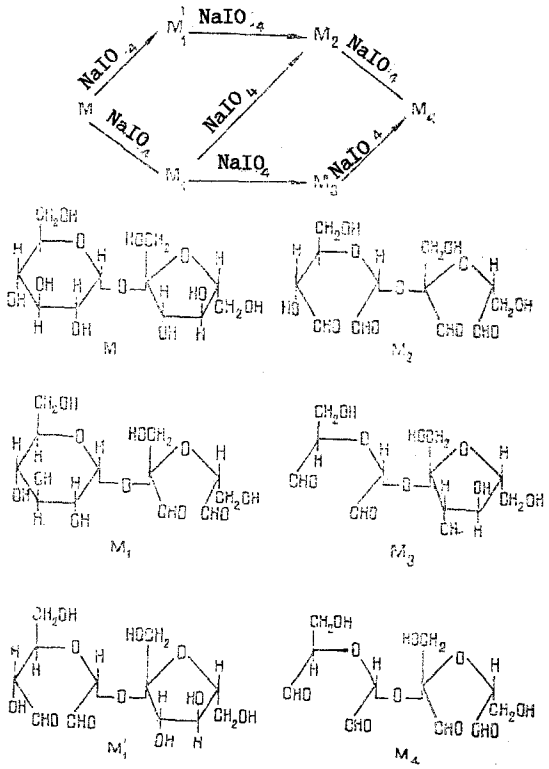

Fig. 2. Scheme of the oxidation of sucrose by sodium periodate. Possible types of products and their designations.

The use for the analysis of oligosaccharides of mass-spectrometric methods with "mild" ionization in step with their development has permitted the elimination of certain difficulties, and, namely, the process of preliminary modification [2-4]. It has been shown that when these methods are used oligosaccharides are represented in mass spectra in the form of quasimolecular ions of the type of $[M + Ct]^+$, where Ct is the cation of an alkali metal or an ammonium ion. The glycosyloxycarbonium ion $[M - 17]^+$ and fragmentary ions formed on the cleavage of glycosidic bonds are also observed in the spectra. In spectra obtained with use

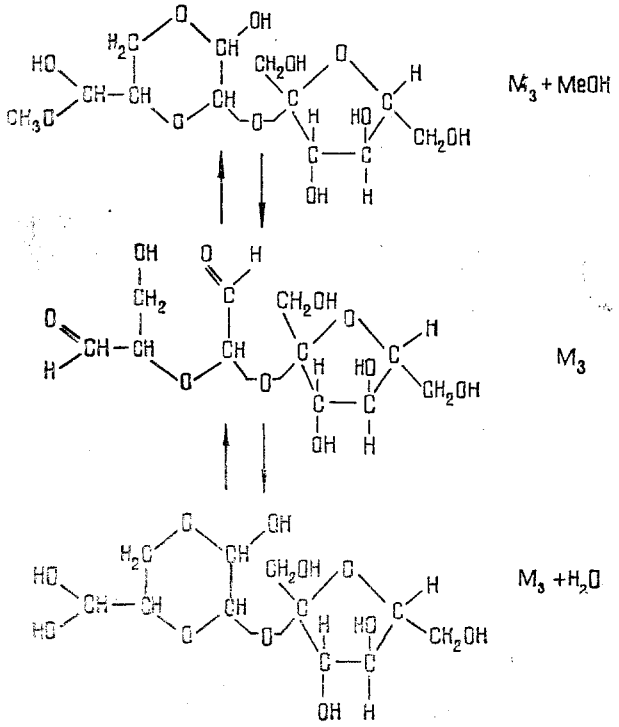

Fig. 3. Scheme of the interaction of one of the forms of oxidized sucrose (M_3) with molecules of a solvent and the OH groups of the initial molecule.

of ionization by fast atoms there are also the cluster ions $[(glycerol)_n + H]^+$, where n reaches 10, which substantially complicates the interpretation of the spectra [3]. A common disadvantage of these methods is the discrete introduction of the sample and the dependence of the type of spectrum on the time of exposure, which prevents the use of such methods for analyzing mixtures of oligosaccharides and excludes an estimation of their relative concentrations.

Free from such disadvantages is the mass-spectrometric method with the electrodynamic spraying of a liquid into a region with an air pressure ensuring evaporation of the solvent. This method has been called EDIAP [Extraction of Dissolved Ions at Atmospheric Pressure] [5]. It has been described in detail in [6]. It is assumed that in this method ions already existing in solution are recorded without any additional action whatever that could decompose the compounds to be analyzed. In actual fact, as has been shown in [7], oligosaccharides obtained on the hydrolysis of laminarin by the endo-1,3 → β-D-glucanase L IV from the marine mollusc Spisula sachalinensis are represented exclusively by the quasimolecular ions $[M + Na]^+$.

In the present paper we give an estimate of the possibility of using EDIAP mass spectrometry for checking the composition of the products formed in the periodate oxidation of sucrose without preliminary fractionation of the reaction mixture. The choice of this reaction as a model is connected with the fact that periodate oxidation recently been used fairly frequently for introducing into a polysaccharide matrix, such as a dextran, aldehyde groups for the subsequent production of prolonged forms of drugs [8-10]. Here, as shown in [11], oxidation should be accompanied by the formation of dialdehydes with different degrees of oxidation of the triol groupings. The biological activity of the preparation depends to a considerable degree on the number of groups introduced into the polymeric matrix [12]. Furthermore, at the present time, as before, periodate oxidation is used to establish the structure of carbohydrates. In this case, the number of triol and glycol groupings is traditionally determined from the consumption of oxidant and the amount of formic acid produced on oxidation.

It is obvious that a strict estimate of the number of groupings oxidized, evem if it is considered that oxidation stops at the stage of formation of dialdehydes, is possible only

TABLE 1. Masses of the Quasimolecular Ions of Sucrose and Possible Products of Its Chemical Modification

Form of the disaccharide M (initial)	$M + Na^+$
Products of the periodate oxidation of sucrose with $NaIO_4$	
$M_1 = M - 2H$	365
$M_2 = M - 4H$	363
$M_3 = M_1 - CH_2O$	361
$M_4 = M_2 - CH_2O$	333
$M_1 + H_2O$	331
$M_2 + 2H_2O$	381
$M_3 + H_2O$	397
$M_4 + 2H_2O$	351
$M_1 + MeOH$	367
$M_2 + 2MeOH$	395
$M_3 + MeOH$	425
$M_4 + 2MeOH$	365
$M_2 + H_2O + MeOH$	395
$M_4 + H_2O + MeOH$	411
	381
Product of the reduction of the oxidized forms of sucrose	
M_1 (+4H)	367
M_2 (+8H)	369
M_3 (+4H)	337
M_4 (+8H)	339

TABLE 2. Results of the Mass-Spectrometric Determination of Sucrose Oxidation Products after Reduction with $NaBH_4$

Form of products	m/z	I (%) at the following ratios of the initial concentrations of sucrose and $NaIO_4$	
		1:1	1:3
M_{init}	365	31	0
M_1 (+4H)	367	100	44
M_2 (+8H)	369	17	70
M_3 (+4H)	337	31	61
M_4 (+8H)	339	13	100

TABLE 3. Concentrations of the Possible Reduced Forms of the Product of the Oxidation Calculated from the Mass Spectra

Form of the product	Yield (%) at following ratios of initial conc. of sucrose and $NaIO_4$	
	1:1	1:3
M_{init}	15,5±0,5	0
M_1	56±2	16±0,5
M_2	9,5±0 5	22±1,5
M_3	15,5±0,5	22±0,5
M_4	7,5±0,5	36±1

when information is available on the purity of the compounds being analyzed and their molecular masses. It may be assumed that on the use of mass-spectrometric detection, together with a determination of the molecular mass of the carbohydrate being analyzed from the difference in the mass numbers between the initial molecule and the products formed, it is possible to determine the numbers and types of glycol groups oxidized if, in the mass spectra, the products arising are recording only in the form of quasimolecular ions.

Since in this method the sample is introduced into the mass spectrometer in the form of a liquid, it may be assumed that the products of the subsequent interaction of the aldehyde groups formed with the molecules of solvent, such as water or methanol, may also be recorded in the spectra [13]. In this method, the time of analysis from the elimination of the solvent to the recording of the compounds is substantially shorter than the time of transition between the various forms, and therefore it may be assumed that the method will permit an estimate of their distribution and relative reactivities. As the subsequent reagents for modifying the oxidation products formed, we have used $NaBH_4$, and NH_2OH. Each of these reagents is widely used in similar reactions; $NaBH_4$, for example, for eliminating an excess of aldehyde groups in polysaccharides after covalent binding with drugs [9, 10], and NH_2OH for determining the concentrations of these groups.

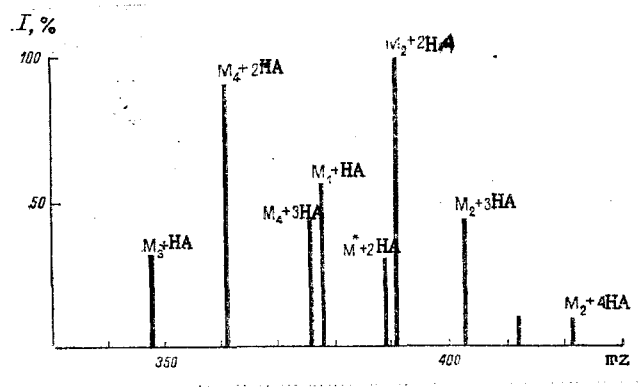

Fig. 4. Mass spectrum of sucrose oxidized with sodium periodate at a ratio of 1:3, and treated with hydroxylamine.

The experiments began with the mass-spectrometric determination of the composition of reaction mixtures obtained at various degrees of oxidation of sucrose. Typical mass spectra of the reaction mixtures after their dilution with methanol in a volume ratio of 1:4 are shown in Fig. 1, a, b. To evaluate the results obtained, let us consider the scheme for the oxidation of sucrose by sodium periodate shown in Fig. 2. This also gives the possible types of products and their designations. According to this scheme, in the oxidation process the formation of at least five types of products is possible, of which two – M_1 and $M_1^!$ – have the same molecular mass. Then the formation of M_2 and M_4 can take place by two routes, while there is only one route for the formation of M_3. As already mentioned, each of the aldehyde groups formed may react further with solvent molecules, and also with the OH groups of the initial partially oxidized sucrose molecule [13]. As an example, Fig. 3 demonstrates possible transformations of M_3, although the scheme does not include other possible types of products that could be formed on ring closure with the participation of other groups, and this all the more because here they will not have different molecular masses. Also omitted from this scheme are completely hydrated forms as being unlikely according to [13]. Since even for such a disaccharide as sucrose the formation of a fairly large number of products is possible, Table 1 gives, in accordance with the designations in Figs. 2 and 3, the values of the molecular masses of the expected quasimolecular ions with Na^+. It follows from a comparison of Fig. 1, a, b and Table 1 that the mass spectra shown contain the expected reaction products, with the exception of the mass values 409, 423, and 441. We may note that, both for sucrose and for the reaction mixtures the spectra do not contain the lines of fragmentary ions in the range of mass numbers from 50 to 300 amu. Each spectrum also contains an intense line corresponding to the Na^+ ion and a less intense line of the K^+ ion, which shows the presence of potassium salts as impurities in the reagents used. Since preliminary results showed that in the method used, just as in other methods with "mild" ionization, the quasimolecular ions with Na^+ possess the greatest intensity [3], the ions with K^+ were not recorded in the spectra. As follows from the results obtained, with an increase in the degree of oxidation the initial form of the sugar disappears and, together with aldehyde forms, methoxy derivatives arise. Although gem-diol forms are present in the spectra – and, for individual products, mixed forms – methoxy derivatives make up the bulk of the products. This shows that in the presence of methanol (in a concentration of 80% by volume) the equilibrium is shifted predominantly in the direction of the formation of methoxy derivatives.

It also follows from an analysis of the results obtained that products of molecular masses of 409, 423, and 441 are formed at high degrees of oxidation. Since the values of the observed ions of 409 and 423 differ from products consisting of possible forms of M_2, it may be assumed that their formation is connected with the oxidation of one of the remaining alcohol groups to an aldehyde group. The appearance of such a (fifth) aldehyde group is accompanied by the addition of another molecule of methanol or water with the formation of a product having a molecular mass of 441 amu.

We may also note that, as mentioned in [13], in actual fact completely hydrated forms or methoxy derivatives are absent from the reaction mixture.

As follows from the figures given in Table 2, on the sodium tetrahydroborate reduction of the products obtained at various degrees of oxidation, the mass spectra are substantially simplified. Even on the addition of $NaBH_4$ in a molar ratio of 1:1 in relation to the initial

sucrose the complete reduction of the aldehydic gem-diol methoxy derivatives to metacetals is observed. For the masses of the quasimolecular forms of the reduced products, see Table 1.

Earlier, as an example of the use of EDIAP mass spectrometry for studying the kinetics of enzymatic reactions [14], the possibility of using mass-spectrometric measurements for evaluating the concentration of the initial substrate formed as the result of the reactions of products having similar values of their molecular masses was demonstrated. If it is assumed that for the reduced forms of the products, as well, the ratios of the intensities correspond to the concentrations of the products formed, it is possible to attempt to estimate their concentrations. Table 3 gives the concentrations of the recorded products calculated on the basis of the mean values in the light of the results obtained in three repeated recordings. It follows from this table that, as was to be expected, with an increase in the amount of oxidant in the reaction the initial form of sucroses disappeared completely. While at a lower degree of oxidation M_1 predominated in the reaction mixture, with an increase in it the maximum concentration was possessed by the product with the highest degree of oxidation, M_4. It is possible that the amount of M_2 at high degrees of oxidation is overestimated, since, on the reduction of the products recorded on oxidation in the form of quasimolecular ions with masses of 409, 423, and 441, products with the same molecular mass as M_2 should have been formed.

Figure 4 shows the mass spectrum of a reaction mixture obtained on the oxidation of sucrose with sodium periodate at a ratio of 1:3 and treated with hydroxylamine for 30 min. According to the literature [13], it may be expected that, regardless of the form in which the aldehyde group obtained on oxidation is present, the formation of oximes in the reaction mixture is possible. In view of the fact that each of the reactions is reversible at the ratios of aldehyde groups formed and hydroxylamine that were used, the spectral characteristics show the formation of products with different numbers of groups converted into oximes. We may note that the initial form of oxidized sucrose and its methoxy derivatives are absent from the spectrum. It may be assumed that this result is the consequence of the high reactivity of the initial aldehyde groups and methoxy derivatives in comparison with intramolecular acetals.

Thus, the possibility has been demonstrated of using EDIAP mass spectrometry for analyzing reaction mixtures obtained in the chemical modification of sucrose. Their analysis, requires 10-200 picomoles, calculated as the initial sample, and the time from the preparation of the sample to the completion of recording is 5 min. The possibility must be particularly mentioned of using the method for monitoring the amounts of products formed as the result of reversible reactions, such as compounds of the type of gem-diols and methoxy derivatives, and also oximes. It is obvious that in view of its rapidity, ease of interpretation, and informativeness, the proposed method can be used successfully for solving other problems connected with the chemistry of oligosaccharides.

EXPERIMENTAL

The following reagents wre used: sucrose, hydroxylamine hydrochloride, and sodium periodate of "ch" ["pure"] grade of domestic production, and Serva sodium tetrahydroborate.

The sucrose was oxidized in aqueous solution at ratios of disaccharide to $NaIO_4$ of 1:1 and 1:3. To 20-ml portions of aqueous solutions of $NaIO_4$ with initial concentrations of $5 \cdot 10^{-3}$ M and $1.5 \cdot 10^{-2}$ M were added 30-mg portions of sucrose. The course of oxidation was followed spectrophotometrically until the $NaIO_4$ had disappeared completely [15].

After the elimination of IO_3^-, 5-ml portions of reaction mixtures containing oxidized sucrose with an initial concentration of $5 \cdot 10^{-3}$ were each treated with 1 mg of $NaBH_4$.

To obtain oximes, 7.3 mg of hydroxylamine hydrochloride was added to 5 ml of an aqueous solution of oxidized sucrose with an initial concentration of $5 \cdot 10^{-3}$ M freed from IO_3^- ions (on the use of the threefold excess of oxidant).

The mass spectra of the reaction mixtures, previously diluted with methanol in a volume ratio of 1:4, were taken on an experimental specimen of the instrumental complex KhZh MS 3303 constructed in the Scientific and Technical Division of the USSR ACademy of Sciences. The rate of feed of the sample to the mass spectrometer was 0.2 μl/min.

SUMMARY

The possibility of using EPIAP mass spectrometry for the rapid determination of the products obtained on the periodate oxidation of sucrose and its subsequent reduction with $NaBH_4$ has been demonstrated. It has been shown that the latter permits the amount of oxidized glycol and triol groupings to be determined and can be used successfully for establishing the structures of carbohydrates. It has also been shown that the method permits the recording directly in the reaction mixtures of the presence of equilibrium forms produced on the interaction of aldehyde groups with molecules of the solvent of hydroxylamine.

Analysis with the aid of EDIAP mass spectrometry requires 100-200 picomoles, calculated as the initial sucrose. The time of analysis is 5 min. The mass spectra contain only the lines of quasimolecular ions of the type of $[M + Na]^+$.

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