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(54) Title: METHOD FOR THE ESTERIFICATION OF CARBOHYDRATES

(57) Abstract

Novel acylated, hydroxyalkylated carbohydrates are described which contain 0.5–2.0 acyl groups and 0.5–4.0 hydroxyalkyl groups on positions other than anomeric positions per monosaccharide unit. A process for the preparation thereof, in particular by enzymatic esterification of a hydroxyalkyl carbohydrate, in particular 2,3–dihydroxypropylsucrose or –lactose, is also described.

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METHOD FOR THE ESTERIFICATION OF CARBOHYDRATES

The invention concerns a process for enzymatically esterifying carbohydrates.

Fatty-acid esters of carbohydrates, both of monosaccharides, disaccharides and oligosaccharides and of polysaccharides, have useful properties such as surfactant, emulsifying and, in some cases, therapeutic properties. For a long time, such esters have usually been prepared by chemical means, in particular by base-catalysed reaction with fatty-acid derivatives.

The chemical esterification of carbohydrates is, however, of low selectivity. In addition, it requires extreme conditions, as a result of which the carbohydrate itself may be affected. As a result, numerous undefined and/or undesirable by-products are produced. Furthermore, chemical treatments are less desirable for foodstuffs applications.

Enzymes such as lipases are also found to be capable of catalysing the esterification of carbohydrates, and specifically with greater selectivity, essentially only primary hydroxyl groups, and under milder conditions. If such an enzyme-catalysed esterification of carbohydrates is carried out in an aqueous medium, the equilibrium is heavily on the hydrolysis side and the yields of esters are consequently low. However, various enzymes, such as lipases and proteases, are also found to be active in organic solvents. Frequently used solvents for enzyme-catalysed esterifications are pyridine, dimethylformamide, tetrahydrofuran and the like. Woudenberg et al. (*Biotechn. Bioeng.* **49**, 328-333 (1996)) have recently described the selective esterification of disaccharides containing C_4/C_{12}- acyl groups under the influence of *Candida antarctica* lipase in tert-butyl alcohol. The esters of maltose and trehalose are obtained with a good yield in this process, but sucrose and lactose are barely esterified under these conditions.

If the known enzymatic esterifications do not therefore always result in the desired yield, an additional disadvantage of these processes is the use of organic solvents. The use of organic solvents is generally accompanied by waste problems and by residues in the esterification product, which is certainly undesirable for nutritional applications. Enzymatic esterification without solvent is successful only for sorbitol and fructose to any usable degree (Ducret et al. *Biotechn. Bioeng.* **48**, 214-221

(1995); Guillardeau et al. *Tenside Surf. Det.* **49**, 342-344 (1992)). Other carbohydrates, especially the larger ones, mix with the fatty-acid derivatives to an inadequate extent, certainly if they have long chains, so that hardly any or no result is obtained.

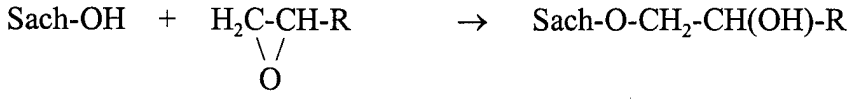
EP-A-268461 discloses a process for preparing fatty-acid esters of epoxyalkyl-substituted sugars, in which an enzymatically obtained epoxyalkyl-glycoside is chemically reacted with a fatty acid. In this process, disaccharides and polysaccharides are cleaved into monosaccharides, such as galactose from lactose. In addition, the epoxide group is always introduced at the anomeric centre.

It has now been found that carbohydrates can be expediently esterified with long acyl groups while retaining the chain length of the carbohydrate if the carbohydrate is chemically modified beforehand by reaction with an epoxyalkane. In the case of short-chain carbohydrates with a DP (degree of polymerization) of up to approximately 4-6, no organic solvent at all is necessary for this purpose. This non-solvent process is suitable for the esterification of monosaccharides and, especially, of disaccharides and oligosaccharides, such as sucrose, lactose, maltose, isomaltose, lactulose, raffinose, stachyose, verbascose, short-chain inulin, amylodextrins and the like, in particular sucrose and lactose. Oligosaccharides are understood to comprise carbohydrates composed of 3-15 monosaccharide units. Sugar alcohols, in particular of disaccharides such as lactitol and maltitol, can also be esterified by this means.

The starting substances for the process may be natural carbohydrates, such as sucrose, lactose, raffinose, starch, inulin, cellulose and the like, or pretreated natural carbohydrates. The possible pretreatment may be, for example, a hydrolysis, in particular an enzymatic hydrolysis, such as a chain shortening of starch (amylase), cellulose (cellulase), hemicellulose (xylanase) and inulin (inulinase). (Semi)synthetic carbohydrates can also be used.

Non-solvent esterification is understood as meaning an esterification in which no appreciable quantities of a non-reacting solvent - as distinct from the fatty acid or its derivative - are used.

The preceding reaction with an epoxyalkane results in a hydroxyalkyl derivative of the carbohydrate. This results in opening of the epoxide ring according to the reaction:



in which Sach represents the carbohydrate residue and R represents hydrogen, alkyl or substituted alkyl, such as hydroxyalkyl, epoxyalkyl, alkoxyalkyl, acyloxyalkyl, alkenyl, mercaptoalkyl, aminoalkyl, haloalkyl, and alkyl especially contains 1-6 carbon atoms. This reaction can take place at any hydroxyl group of the carbohydrate. This reaction is fundamentally different from the enzymatic condensation reaction with glycidol according to EP-A-268461, in which the carbohydrate reacts with the hydroxyl group of glycidol retaining the epoxide ring, at the anomeric centre, where the hydroxyl group is part of a cyclic hemiacetal function and is thus in equilibrium with an aldehyde or ketone. This hydroxyalkylation may take place in a manner known per se, for example by reaction of the carbohydrate with an excess of epoxyalkane, in the presence of a base, such as sodium hydroxide, sodium bicarbonate, potassium carbonate or triethylamine. Said hydroxyalkylation essentially takes place at other sites than an anomeric centre that may be present. A monofunctional epoxide, such as ethylene oxide or propylene oxide, can be used as epoxyalkane, but the epoxide preferably also has a second function, such as a hydroxyl, alkoxy, epoxy, amino or thiol group, which can later be acylated. Examples are glycidol, glycidyl ethers, epichlorohydrin and butanediepoxide.

By way of example, the following general process can be specified for the preceding reaction with an epoxyalkane. 100 to 800 mmol of glycidol are added to 100 mmol of carbohydrate (monosaccharide units) in 100 ml of basic water (for example, 0.01-1 M hydroxide solution). After a few hours (normally 1 to 5 hours, depending on temperature, normally between 40 and 100°C), the reaction is complete and the reaction mixture is neutralized. The mixture is evaporated and is ready for enzymatic esterification.

In the case of hydroxyalkyl carbohydrates, the final esterification can be done chemically or enzymatically. The chemical esterification takes place in the standard manner by reaction of a reactive derivative of the fatty acid, such as the chloride, the anhydride or an alkyl ester, normally in excess, in the presence of a base and possibly a catalyst. Preferably, however, the esterification takes place enzymatically.

The enzymatic esterification is done with a long fatty acid or a derivative

thereof, such as an ester. In this connection, the ethyl ester is preferred because, unlike, for example, the methyl ester, it does not have an interfering action on the esterification enzyme. In general, the process is suitable for introducing acyl groups, in particular acyl groups containing at least 6 carbon atoms, the preference being for acyl groups having at least 10 carbon atoms and up to approximately 24 carbon atoms. A product having usable properties is obtained even with an average of 0.2 long acyl groups per monosaccharide unit or with at least one long acyl group per molecule. In particular, a product containing 0.5-1.0 C_6-C_{24}-acyl groups per monosaccharide unit is prepared. In the case of disaccharides, for example, a mono-C_6-C_{24}-acyl or a di-C_6-C_{24}-acyl derivative or a mixture thereof, optionally with higher esters, is prepared. The long acyl groups are essentially introduced at the primary positions, in the case of sucrose first at position 6 of the fructose unit. In the hydroxyalkyl carbohydrates, the acyl groups can also be introduced on the hydroxyalkyl group.

In the enzymatic esterification, a suitable enzyme is used, in particular a lipase. An example of a usable lipase is *Candida antarctica* lipase, which is commercially available under the name Novozym 435. Other lipases, for example those of other species of the genus *Candida*, or of the genera *Mucor, Rhizopus, Chromobacterium, Pseudomonas* and other ones are also suitable. Other enzymes, including certain proteases such as subtilisin, can also be used for the enzymatic esterification. The enzymes may or may not be immobilised.

If a solvent is used for the enzymatic esterification, it may be a polar organic solvent, such as an alcohol, ether, ketone, amide, nitrile, chloride, sulphone, sulphoxide or nitro compound. Preferably, a tertiary alcohol, such as tert-butyl alcohol, or for example dichloromethane or dimethylformamide is used. Preferably, little or no water is present. Water can be removed during the reaction, for example by using vacuum, using an azeotrope such as hexane/t-butyl alcohol, or adding silica-gel or molecular sieves.

The invention also relates to acylated hydroxyalkylated carbohydrates which contain 0.5-2.0 acyl groups and 0.5-4.0 hydroxyalkyl groups per monosaccharide unit. In this connection, the acyl groups may be esterified both with hydroxyl groups of the carbohydrate itself and with hydroxyl groups of the hydroxyalkyl groups. The esters according to the invention thus conform to the formula:

$(Acyl)_{n-p}Sach(Hyal)_{m-p}(HyalAcyl)_p$,

in which Sach is the carbohydrate radical, Acyl is the acyl group and Hyal is the hydroxyalkyl radical, m is the total number of hydroxyalkyl groups coupled to the carbohydrate, n is the total number of acyl groups coupled to the carbohydrate and p is the number of acyl groups coupled via a hydroxyalkyl group to the carbohydrate, in which connection p may be an integer between 0 and the smaller of m and n. Two or more hydroxyalkyl groups may also be coupled to one another, as in the case where 2,3-dihydroxypropyl groups yield the structure Sach-O-(CH_2-CHOH-CH_2O)-(CH_2-CHOH-CH_2O)$_q$-Acyl, where q is 0-10, in particular 0-2.

The acyl groups may, for example, be alkanoyl, alkenoyl, alkynoyl, alkapolyenyl, aroyl, arylalkanoyl or cycloalkylalkanoyl groups or substituted variants thereof, such as groups substituted with hydroxyl, alkoxy, carboxyl, amino, alkoxycarbonyl and the like. Examples are acetyl, propionyl, caproyl, decanoyl, lauroyl, palmitoyl, stearoyl, ricinoleoyl, acryloyl, propiolyl, oleoyl, linoleyl, benzoyl, phenylacetyl, p-methoxycinnamoyl, nicotinoyl, cyclohexylcarboxyl, camphoroyl, and mixtures thereof, such as mixtures of C_{10}-C_{18}-acyl groups. The preference is for C_6-C_{20} alkanoyl groups. The hydroxyalkyl groups may be hydroxyethyl, hydroxypropyl, 2,3-dihydroxypropyl, 2,3,4-trihydroxybutyl and the like, in particular 2,3-dihydroxypropyl, in which connection said groups may be acylated in addition to or instead of the hydroxyl groups of the carbohydrate itself.

The invention furthermore relates to novel intermediates in the form of hydroxyalkylated disaccharides or polysaccharides which contain 0.5-4.0 2,3-dihydroxyalkyl groups or other polyhydroxyalkyl groups, essentially or exclusively at positions other than any anomeric position present, per monosaccharide unit. The disaccharides or polysaccharides are, in particular, sucrose, lactose, maltose, isomaltose, cellobiose and higher homologues, such as maltotriose, kestose and raffinose.

The invention yields surfactant and emulsifying compounds which are, on the one hand, obtained from renewable raw materials, namely sugars, glycerol and fatty acids, and which, on the other hand, are biologically degradable. As a result, they are environmentally friendly both as regards the production and as regards the final processing. In addition, the substances are suitable for use in foodstuffs.

Examples

Example 1

Etherification of sucrose with glycidol

Ten g of sucrose (29.2 mmol) are dissolved in 20 ml of water, to which 29 mmol of sodium hydroxide (0.15 M) is added as a catalyst/initiator. To the clear solution 1-6 equivalents of glycidol (with respect to sucrose) are added dropwise at 80°C. After stirring for 2 hours, the reaction mixture is cooled and neutralized with dilute (! M) hydrochloric acid. The reaction mixture is then concentrated by evaporation. The reaction is analysed by GC. The results are summarized in table 1.

If the reaction of sucrose and glycidol is carried out with 1.5 M sodium hydroxide solution, the main reaction is the formation of glycerol.

Table 1
Product distribution glyceryl-sucrose (Gly = glyceryl)

number of equiv. glycidol	molar percentage sucrose	molar percentage sucrose-Gly_1	molar percentage sucrose-$Gly_{\geq 2}$
1	58	31	11
2	43	42	14
3	30	51	19
4	17	39	45
6	0	8	92

In all the reactions described above, glycerol is a by-product. Since it would yield glycerol esters as a by-product in the esterification step, the glycerol is removed by means of column chromatography using a column of Norit ROX 0.8. Glycerol is eluted with water, after which sucrose and coupling products of glycidol and sucrose are eluted with a gradient of acetone in water (up to 10%).

Example 2

Enzymatic esterification of glycerylsucrose with ethyl palmitate

Glycerylsucrose (2 g), which has been synthesized using 3 equivalents of glycidol and purified from glycerol (see Example 1), is suspended in 10 ml of tert-

butyl alcohol and 4 g of ethyl palmitate. After 1 g of zeolite 5A has been added to the suspension to remove water and ethanol, the reaction is heated to reflux and stirred. The reaction is initiated with 100 mg of immobilized lipase from *Candida antarctica*. After 24 hours, the conversion is approximately 30%, with monoesters of glycerylsucrose as the main product.

Example 3

Enzymatic esterification of glycerylsucrose with ethyl palmitate without solvent

Glycerylsucrose (20 g) was mixed with 20 g of silicagel. Ethyl palmitate (40 g) and 2 g of lipase (*C. antarctica*) were added to the homogenised mixture. The suspension was stirred under vacuum for 5 days at 70°C. After work-up (filtering, washing with methanol, water and methanol, and evaporating), 44 g of crude product was obtained, from which 3.45 g of glyceryl-sucrose palmitate was isolated after column chromatography. Melting point with DSC: from 41.9 to 58.8°C.

Example 4

Etherification of sucrose with allyl glycidyl ether

Sucrose (1 g, 2.9 mmol) was dissolved in 10 ml of water, to which 1.5 ml 2 M sodium hydroxide solution was added at 70°C, 1.2 ml allyl glycidyl ether (10.2 mmol) was slowly added dropwise, and stirring was continued for another 2 hours at 70°C. After neutralization and evaporation, the product composition was determined chromatographically (molar basis): 21% sucrose, 40% sucrose mono(allyloxy-hydroxypropyl) ether, 19% sucrose diether, 9% sucrose triether and 2% sucrose tetraether, in addition to a substantial amount of allyl dihydroxypropyl ether.

Example 5

Etherification of inulin with glycidol

Fifty g of inulin (average chain length 8 monosaccharide units; about 300 mmol) was dissolved in 500 ml of water. 29 ml of 1 M sodium hydroxide was added. The solution was heated to 70°C, and then 45 ml of glycidol (50 g, 680 mmol) was added in two hours. Stirring was continued at this temperature for another two hours, followed by a neutralization of 1 M hydrochloric acid. The solution was concentrated

by evaporation and mixed with 150 g silicagel for purification. The mixture was transferred to a glass filter and washed with ethylacetate/isopropanol/water (65:22.5:12.5), resulting in removal of all glycerol. The glycerol-inulin was then washed from the silicagel with methanol (1 l). Yield of glycerol-inulin 55 g.

Example 6

Oil-in-water emulsion

Oil was carefully added to 20 g of water with 0.5 wt.% of glyceryl-sucrose palmitate using an ultra-turrax. A thick emulsion was obtained. 150 g of oil could be added without the emulsion being broken. After two months, an emulsion of 1:5 water:oil had not broken yet, even though a small amount of oil was visible on top of the emulsion.

Example 7

Water-in-oil emulsion

Following the process of EP-A-506352, a water-in-oil emulsion was prepared by adding 2 g glyceryl-sucrose palmitate and dispersing using an ultra-turrax, adding 50 ml of oil and stirring at 300 rpm with an ultra-turrax for three minutes. Pouring into a calibrated cylinder results in phase separation, however much later than in case of a comparable water-in-oil emulsion to which Sisterna SP-70 was added as an emulsifier.

Claims

1. An acylated, hydroxyalkylated carbohydrate which contains 0.5-4.0 hydroxyalkyl groups at non-anomeric positions and 0.5-2.0 acyl groups per monosaccharide unit.

2. An acylated, hydroxyalkylated carbohydrate according to Claim 1, wherein the acyl groups are C_6-C_{20}-alkanoyl groups.

3. An acylated, hydroxyalkylated carbohydrate according to Claim 1 or 2, wherein the hydroxyalkyl groups are optionally acylated 2,3-dihydroxyalkyl groups.

4. A hydroxyalkylated monosaccharide, disaccharide or polysaccharide which contains 0.5-4.0 2,3-dihydroxyalkyl groups at non-anomeric positions per monosaccharide unit.

5. A process for esterifying carbohydrates with a fatty acid or an active derivative thereof, characterized in that the carbohydrate is hydroxyalkylated with an epoxyalkane prior to the esterification.

6. A process according to Claim 5, wherein 0.2-4.0 hydroxyalkyl groups, in particular 2,3-dihydroxypropyl groups, are introduced per monosaccharide unit.

7. A process according to Claim 5 or 6, wherein 0.5-1.0 C_6-C_{20}-acyl groups are introduced per monosaccharide unit.

8. A process according to one of Claims 5-7, wherein the esterification is carried out enzymatically, preferably with an ethyl ester of the fatty acid.

9. A process according to one of Claims 5-8, wherein the carbohydrate is a monosaccharide, disaccharide or oligosaccharide, in particular sucrose or lactose.

10. A process according to one of Claims 5-9, wherein the esterification is carried out without solvent or with a tertiary alcohol as solvent.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H15/26 C07H15/06 C07H3/04 C07H3/06 C08B11/08
C08B31/12 C08B37/18 C12P19/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H C08B C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	EP 0 268 461 A (NOVO INDUSTRI AS) 25 May 1988 cited in the application see the whole document ---	1-3,5-7
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

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