

PATENT SPECIFICATION

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(54) NEW AMINO-SUGAR COMPOUNDS, THEIR PRODUCTION, AND THEIR MEDICINAL USE

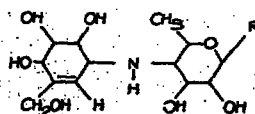
(71) We, BAYER AKTIENGESellschaft, a body corporate organised under the laws of Germany, of Leverkusen, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to certain new amino-sugar compounds, to their preparation, and to their medicinal use, especially as agents for the control of carbohydrate metabolism and against diabetes and adiposity.

It has already been disclosed that a number of the micro-organisms *Actinomycetes*, above all those of the family *Actinoplanaceae*, for inhibitors for glycoside-hydrolases, especially for carbohydrate-splitting enzymes of the digestive tract. Some of these inhibitors are relatively heat-stable and are also stable to acid and alkali at room temperature. Chemically, these inhibitors are oligosaccharides, polysaccharides or derivatives thereof.

Most of the inhibitors from this group are very potent amylase inhibitors but only medium- or week-strength saccharase inhibitors (see German Offenlegungsschrift (German Published Specification) 2,064,092).

This invention now provides new compounds which are amino-sugar derivatives of the following general formula:—



(I)

in which R is an oligosaccharide chain with one, two, three or four (preferably one, two or three) mono-saccharide units, the said compounds being substantially free from homologous amino-sugar derivatives containing more than four (preferably three) mono-saccharide units per molecule.

The monosaccharide units in R are preferably hexose residues, especially glucose residues.

The invention further provides a process for the production of an amino-sugar derivative in which either:

(a) a micro-organism of the strain *Actinoplanes* SE 50/110 (CBS 674.73) is cultured in an aqueous medium under aerobic conditions and a compound of formula I or a homologue thereof containing up to seven monosaccharide units in the side chain is separated from the resultant culture; or

(b) a compound of the invention of the general formula I is produced from a homologous amino-sugar derivative containing up to seven side-chain monosaccharide units by splitting off the necessary number of monosaccharide units from the molecule microbially, or by chemical or enzymatic hydrolysis and

freeing the resulting material from homologous amino-sugar derivatives containing more than four monosaccharide units per molecule.

The compounds of the invention are powerful inhibitors of glycoside-hydrolases of the digestive tract. The lower members, in which R is an oligosaccharide chain with 1 or 2 hexose units, predominantly exhibit powerful inhibition of saccharase and the higher members predominantly powerful inhibition of amylase.

Surprisingly, the compounds of the invention possess a greater specific inhibitory activity against saccharase or amylase than the inhibitors already known. An especially peculiar feature which is conspicuous is that some of the new compounds possess an inhibiting action *in vivo* which is many times greater than their inhibiting action *in vitro*.

Furthermore, it must be regarded as particularly surprising that the lower members of the homologous series according to the invention inhibit glucose resorption *in vivo*.

Because of these surprising properties, the new compounds of the invention represent an enrichment of the range of available pharmaceuticals.

In order to prepare compounds of the formula I in crude form a micro-organism of the family *Actinoplanaceae* (belonging to the order *Actinomycetales*), and preferably a strain of the genus *Actinoplanes*, are used. Examples which may be mentioned are the strains *Actinoplanes* spec. SE 50 (CBS 961.70), SB 18 (CBS 957.70) and SE 82 (CBS 615.71). These micro-organisms have already been described in South African Patent No. 71/8,677 and have been deposited, under the numbers shown in parentheses, in the Centraalbureau voor Schimmecultures in Baarn/Holland.

Furthermore mutants or variants of these strains can also be used for carrying out the process according to the invention — see, again, South African Patent No. 71/8,677. The strains SE 50/13 (CBS 614.71) and SE 50/110 (CBS 674.73) have proved to be particularly suitable with regard to the total yield of the amino-sugar derivatives according to the invention and/or with regard to the size of the radical R in the mixture of the amino-sugar derivatives formed in the fermentation. The description of both strains corresponds largely to that of the parent strain SE 50 (CBS 971.60). These strains have been obtained by natural selection from strain SE 50, (CBS 971.60), without using mutagens.

The microorganisms may be cultured in a solid or liquid, but especially liquid, aqueous nutrient media, which as usual, in addition to containing sources of carbon, contain sources of nitrogen, salts and anti-foaming agents in the customary concentrations. The concentrations can vary within wide limits.

The carbon sources used are generally carbohydrates, especially starch, maltose, glucose and mixtures of two [or three] of these materials; complex mixtures, for example, commercially available malt extract, may also be used.

The nitrogen sources which can be used include the complex mixtures customary in microbiology, for example, casein hydrolysate, yeast extract, peptone, fishmeal, fish solubles, corn steep liquor, meat extract and mixtures thereof, as well as aminoacids and/or ammonium salts.

The cultivation is in general carried out aerobically in aerated shaken cultures or in container cultures. The nature and concentration of the source of carbon, in combination with the particular strain used for the fermentation, influences the nature of the end product, with regard to the size of the radical R, to the extent that individual members of the homologous series or at least a very narrow range of homologues can be prepared preferentially and indeed almost selectively.

Thus we have found that in nutrient solutions which contain more than 2 wt. % of starch, compounds of the invention with 4 hexose units are predominantly formed along with homologues thereof containing 5, 6 and 7 hexose units, the strain SE 50 (CBS 961.70), and the strain SE 50/13 (CBS 614.71) in particular, being suitable for this production. However, under certain circumstances it suffices merely to add 0.1–3 wt. %, preferably 0.5–2 wt. %, of starch to nutrient solutions which contain adequate glucose (e.g. about 3.5 wt. %) as the base source of carbon, in order to obtain mixtures of compounds of the invention with 4–7 hexose units.

In addition it has been found that in starch-free solutions and especially on addition of maltose to the nutrient solution, especially when using strain SE 50 (CBS 961.70), compounds of the invention with 2–3 hexose units are predominantly formed.

Nutrient solutions which contain only glucose as the source of carbon have

proved particularly suitable for preparation of the compounds of the invention in which R consists of one glucose residue.

If the nutrient solution contains excess glucose, the longer-chain compounds (i.e. compounds containing 4—7 glucose residues) are also formed if the duration of fermentation is prolonged. This can be prevented, within certain limits, by making the exhaustion of the nitrogen sources during fermentation coincide in time with the exhaustion of the glucose. If the glucose is entirely dispensed with in the nutrient solutions and maltose is added as the source of carbon, the compound with 2 hexose units is obtained predominantly. It is also possible to replace the pure maltose by cheaper mixtures such as, for example, Maltzin, a natural malt extract from Diamalt AG, Munich. Corresponding to the content of maltotriose, the next-higher homologue is then also formed at the same time.

The strain SE 50/110 (CBS 674.73) has proved to be particularly suitable for the preparation of the lower homologues (i.e. containing 1, 2 or 3 monosaccharide units); in optimal nutrient solutions this strain gives a yield of lower homologues about twice that given by the strain SE 50/13 (CBS 614.71).

During the fermentation reactions the composition of the remaining nutrient solution, and especially the concentration of the nitrogen sources and the salt composition, can vary within wide limits.

The nutrient media used can be sterilised in the usual manner. The pH values of the nutrient solution generally lie between 5.0 and 8.5, preferably between 6.0 and 7.8. The incubation temperatures generally lie between 15 and 45°C, preferably between 24 and 32°C. For the preparation of higher homologues (i.e. compounds containing 4—7 monosaccharide units) SE 50 (CBS 961.70) and SE 50/13 (CBS 614.71) a higher temperature, for example 28°C, is more advantageous whilst for the preparation of the lower homologues (i.e. containing 1, 2 or 3 monosaccharide units) using the strains SE 50 (CBS 961.70) and SE 50/110 (CBS 674.73), a lower temperature, for example 24°C, is more favourable. The duration of culture is generally 1—8 days, preferably 2—6 days. Longer durations of culture, especially if an excess of carbohydrate is used, favour the formation of the higher homologues. The end point of the fermentation can be determined by determining the inhibitory activity content in an enzymatic inhibition test and by determining the composition by thin layer chromatography.

The lower homologues of the series forming the compounds of the invention can also be obtained from the higher homologues by splitting off monosaccharide units. The splitting off can be carried out by chemical or enzymatic hydrolysis or microbially.

Chemical hydrolysis is preferably carried out in 1—5N aqueous mineral acid at 50—100°C, especially at 90—100°C, over a period of 10—180 minutes.

Enzymatic hydrolysis is preferably carried out by incubation with a suitable hydrolase, especially a β -amylase, an α -amylase that is not inhibited by the compounds of the invention, or an amyloglucosidase of microbial origin (e.g. from *B. subtilis*).

The enzymatic hydrolysis is carried out microbially preferably by culturing a suitable micro-organism in a nutrient medium containing 1—10% of the compound of the invention which is to be split as sole carbon source. Suitable micro-organisms include, for example, *Aspergillus niger* ATCC 11,394.

The isolation and purification of the compounds of the invention starts either from microbiological culture broths or from acid hydrolysates or from incubation mixtures in which the enzymatic and/or microbiological restructuring or degradation of the higher homologues of the amino-sugar derivatives has been carried out.

Various methods of working up become necessary depending on the molecular weight range of the compounds which are to be isolated.

The higher homologues (i.e. containing 4—7 monosaccharide units) can be initially separated by direct precipitation after prior decolourising and concentrating of the solutions.

In contrast, the lower-molecular homologues (containing 1—3 monosaccharide units) are preferably initially separated by adsorption on active charcoal at a neutral pH, with subsequent desorption with aqueous alcohols or acetone, preferably 50—80% strength acetone. The desorption can be carried out particularly completely at acid pH values in the range of pH 1.5—4; preferably pH 2—3.

If the starting solutions are very dark in colour, they are decolourised, prior to the adsorption of the compounds of the invention, by means of active charcoal at

acid pH values (pH 1—3) or with unspecific adsorption resins, for example Lewapol Ca 9221/0.35 mm particle size (Bayer AG) in the pH range of 2—7, preferably at pH 2—3. The active charcoal preferentially binds dyestuffs in the acid range only, whilst Lewapol does not adsorb the amino-sugar derivatives according to the invention, which act as inhibitors, either in the neutral or in the acid range.

In order to separate the compounds of the invention from inactive saccharides and other inactive compounds which also have inhibiting action, the weakly basic character of the compounds of the invention can be utilised. Under suitable conditions (pH 1—8, preferably pH 2—4; at low ionic strength, corresponding to a conductivity of less than 10 mS, preferably less than 2 mS), the compounds of the invention are bound by strongly acid cation exchangers such as, for example, Dowex 50 W (Messrs. Dow Chemicals) in the H^+ form. We have found that the compounds of the invention can be bound particularly successfully from acetone solution (50—80% acetone, pH 1—5, preferably pH 2—4) to cation exchangers, which under these conditions exhibit a substantially enhanced adsorptive capacity for the compounds. Provided the solution contains more than 50% of acetone it is also possible to bind the compounds of the invention very well to weakly acid exchangers such as, Amberlite IRC—50 (H^+ form).

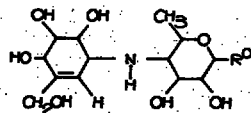
Aqueous solutions of acids or bases, preferably ammonia or hydrochloric acid, particularly in concentrations of 0.01—1 gram-equivalents/litre are best used for desorbing the compounds of the invention from the cation exchangers.

The desorbates are neutralized with a weak acid respectively basic ion exchanger or the base respectively acid is stripped from the desorbates *in vacuo* and the compounds of the invention are obtained, after concentrating the solution, by lyophilisation or by precipitation with organic solvents, preferably with 10—20 volumes of acetone.

Furthermore, it has proved possible to separate the low-molecular compounds of the invention from inert saccharides by chromatography on exchangers based on cellulose, preferably phospho-cellulose (Messrs. Serva, Heidelberg). Buffers, preferably phosphate buffers, of low ionic strength, preferably 2—100 mM, especially 5—10 mM, in the pH range of 2.5—8, preferably at pH 5—6, are preferably used as running agents. The prerequisite for effective fractionation is that the salt contents in the preparations to be fractionated should be as low as possible.

To prepare in a pure state the individual compounds of the invention, the pre-purified preparations prepared as described above can be chromatographed using a suitable molecular sieve, for example Bio-Gel P—2 (Messrs. Bio-Rad, Munich), and the fractions of the eluate examined by thin layer chromatography. Fractions which contain the pure compounds of the invention are combined, re-chromatographed and finally lyophilised after concentration, or precipitated by means of organic solvents, as described above.

The compounds of the invention are, chemically, carbohydrates. They form homologous series of which the following amino-sugar derivative [$C_{19}H_{33}O_{13}N$] is to be regarded as the base substance:

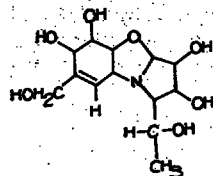


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[R° = a single monosaccharide, pref. hexose, esp. glucose, residue].

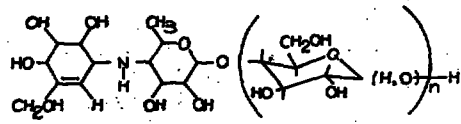
The higher members are derived from the base substance through successively containing one additional unit of a monosaccharide, preferably of a hexose, especially of glucose.

All members of this homologous series are further characterised in that on acid total hydrolysis they give "component I" [$C_{13}H_{19}O_8N$] as well as the corresponding monosaccharide. The structural formula shown below was deduced for "component I":—



"Component I"

This compound has a strong anti-hyperglycemic action.
Formula Id represents the compounds of the invention containing one or two glucose units:—



Id

5 The base substance of this series is that wherein $n = 1$ and is described herein as "component II". Each member of the series differs from the next lower or next higher member through having an additional glucose unit or a missing glucose unit. 5

10 The compound which is larger than "component II" by one glucose unit (i.e. in which $n = 2$) is designated "component III", the compound which is larger by 2 glucose units (i.e. $n = 3$) is designated "component IV", and so on. 10

All these compounds are characterised in that on acid total hydrolysis they give "component I" and glucose.

15 "Component II" can be characterised as follows with respect to its physico-chemical properties, its structure and its reactivity: 15

Component II is a colourless, amorphous solid substance of good solubility in H_2O , DMF, DMSO and MeOH. It is also soluble in hot ethanol.

20 a) Thin layer chromatography:
Running agent: ethyl acetate/MeOH/ H_2O , 10:6:4 (v/v)
("TLC" = Thin Layer Chromatography") 20

I. F 1,500 silica gel ready-to-use TLC films from Messrs. Schleicher & Schüll

- Rf-values: II 0.46
- Maltose: 0.50
- Glucose: 0.65

25 II. F 254 silica gel ready-to-use TLC plates from Messrs. Merck. 25

- Rf-values: II 0.47
- Maltose: 0.54
- Glucose: 0.66

30 $AgNO_3/NaOH$ spray reagent is best used for rendering II visible on silica gel plates. A brown-black coloration is obtained even at room temperature or after slight warming. 30

b) Gas chromatography:

35 For examination by gas chromatography, II is silylated together with α - and β -D-glucose or sucrose as the internal standard, in a mixture of pyridine (1), trimethylchlorosilane (0.5) and N-methyl-trimethyl-silyl-trifluoroacetamide (1). 35

Column: 6 ft glass filled with 3% SE 30 on Chromosorb WAW ("Chromosorb" is a Registered Trade Mark; SE 30 = silicon-elastomer of Hewlett Packard)

40 Temperatures: Injection block 300°C
Detector 300°C 40

Oven: 220°C, isothermal, until elution of the peaks of α - and β -D-glucose; subsequent temperature programme 15°C/minute up to 300°C.

Detector: FID (FID = flame ionization detector)

Gases: Carrier gas: N₂ 40 ml/minute
 Combustion gas: Air 80 ml/minute
 H₂ 20 ml/minute

Retention times: α -D-glucose 3 minutes
 β -D-glucose 4 minutes
 Sucrose 12–13 minutes
 II 16–17 minutes

c) Optical rotation:

A non-crystalline sample of II obtained by concentrating a solution of II in methanol was dissolved in water and the specific optical rotation was found to be $[\alpha]_D^{20} = +134.3^\circ$.

d) IR spectrum:

Rather inconclusive IR spectrum, of poor structure, in KBr. Main absorption bands in the range of the O—H and C—O valency vibrations.

e) NMR spectrum in CD₃OD at 220 MHz: (Figure 1)

in ppm	Multiplicity	Relative intensity
1.3	Doublet; J = 6.5 Hz	3 H
2.3	"Triplet", J ₁ and J ₂ 8–10 Hz	1 H
3.15	"Triplet", J ₁ and J ₂ 7–9 Hz	1 H
3.3 – 3.9	Signals cannot be allotted individually	12 H
4.13	AB system; J = 12 Hz	2 H
4.48 } and 5.1 }	Doublet; J = 7 Hz } Doublet; J = 2.5 Hz }	1 H
4.9	Singlet	11 H protons replaced by deuterium
5.0	Doublet; J = 2–3 Hz (poor resolution)	1 H
5.8	Doublet; J = 3–4 Hz (poor resolution)	1 H
		33 H

(Abscissa in the figures = δ (ppm))

f) Acetylation of II:

II is reacted in a 1:1 mixture of acetic anhydride and pyridine at room temperature to give a decaacetyl derivative (molecular weight: 903). If the reaction is carried out in 1:1 glacial acetic acid/acetic anhydride with catalytic amounts of H₂SO₄, the formation of an undecaacetyl derivative (molecular weight: 945) can also be detected by mass spectroscopy alongside the decaacetyl derivative.

NMR spectrum of the decaacetyl derivative: (Figure 2)

MS spectrum of the decaacetyl derivative:

Molecular peak: 903 (2.5% relative intensity)

Base peak: 843

Important fragment peaks in the upper mass range:

844 (55% relative intensity)

784 (36% relative intensity)

783 (34% relative intensity)

759 (35% relative intensity)

556 (36% relative intensity)

496 (37% relative intensity)

436 (29% relative intensity)

g) Methylation of II

The methylation of II with $\text{CH}_3\text{I}/\text{NaH}$ in dimethyl sulphoxide by the Hakomori method gives, as the main product, the decamethyl derivative of II and in small amounts also the undecamethyl derivative.

Mass spectrum:

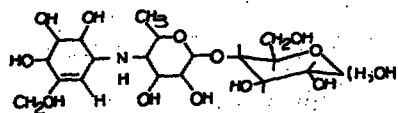
Molecular peak: 623 (6.1% relative intensity)

Base peak: 535

2nd molecular peak: 637 (0.2% relative intensity)

Spectroscopic data and chemical properties show the following structure for

II:



II is a mixture of an α - and a β -form, as is shown, in particular, by the NMR spectra.

The next member of the homologous series "component III" [$\text{C}_{22}\text{H}_{43}\text{O}_{18}\text{N}$], can be characterised as follows with regard to its physico-chemical properties, its structure and reactivity.

III is a readily water-soluble amorphous solid product.

a) Thin layer chromatography:

Running agent: ethyl acetate/ $\text{MeOH}/\text{H}_2\text{O}$, 10:6:4 (v/v)

I. F. 1,500 silica gel ready-to-use TLC films (Schleicher & Schüll):

R_f-values: III: 0.35
Maltose: 0.50

II. F 254 silica gel ready-to-use TLC plates (Merck):

R_f-values: III: 0.33
Maltose: 0.54

III gives a brown-black colouration with the $\text{AgNO}_3/\text{NaOH}$ spray reagent even at room temperature or on slight warming of the plates.

b) IR spectrum:

Rather inconclusive IR spectrum, of poor resolution, in KBr. Main absorption bands in the range of the O—H and C—O valency vibrations. (3,700—3,100 cm^{-1} and 1,180—950 cm^{-1} , respectively).

c) Optical rotation:

$[\alpha]_D$ in H_2O : + 147.2°

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d) NMR spectrum of III in D₂O at 220 MHz: (Figure 3)

e) Methylation by the Hakomori method:

Methylation of III with CH₃I and NaH in dimethylsulphoxide gives, as the main product, III methylated 13-fold, and also small amounts of the product which has been methylated 14-fold.

f) Mass spectrum of the methylation product:

The molecular peak at 827 (1.5% relative intensity) corresponds to an empirical formula C₃₈H₆₉NO₁₈. (A second molecular peak of 0.1% relative intensity is present at 841).

The most important fragment peaks are:

739 (27% relative intensity)

592 (3.7% relative intensity)

535 (30% relative intensity)

388 (9% relative intensity)

386 (13% relative intensity)

284 (13% relative intensity)

187 (12% relative intensity)

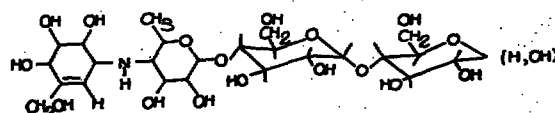
171 (40% relative intensity)

101 (34% relative intensity)

88 (25% relative intensity)

75 base peak

The following structure of component III is in accord with all chemical and spectroscopic properties:



Component IV is the next higher member of the homologous series. On acid partial hydrolysis, the compound can be split to give component II and glucose in the molar ratio of 1:2.

Thin layer chromatography:

Running agent: n.-butanol/ethanol/water = 50:30:20

F 1,500 silica gel ready-to-use TLC plates (Schleicher & Schüll):

R_{Glucose}-values: IV: 0.41—0.46

The higher homologues, especially components V—VIII, are very much less able to inhibit saccharase and very much more able to inhibit α-amylase from the pancreas, than the lower members of the series (components II—IV).

On acid hydrolysis of the higher components, the corresponding lower components can in each case be detected as intermediate products; in addition, glucose and maltose are formed as products of the splitting reaction.

Thin layer chromatography:

Running agent: n.-butanol/ethanol/water = 50:30:20 (v/v)

F 1,500 silica gel ready-to-use TLC plates (Schleicher & Schüll):

R_{Glucose}-values: V: 0.30—0.34

VI: 0.21—0.23

VII: 0.14—0.16

VIII: 0.09—0.11

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The specific inhibitory activities of the compounds of the invention can be determined by means of *in vitro* enzyme inhibition tests.

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Amylase test

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One amylase inhibitor unit (1 AIU) is defined as the amount of inhibitor which inhibits two amylase units to the extent of 50%. One amylase unit (AU) is the amount of enzyme which under the test conditions specified below splits 1 μ equivalent of glucoside bonds in the starch per minute. The μ equivalents of split bonds are determined colorimetrically as μ equivalents of reducing sugars formed, using dinitrosalicylic acid, and are quoted as μ equivalents of maltose equivalents, using a maltose calibration curve. To carry out the test, 0.1 ml of amylase solution (20—22 AU/ml) are mixed with 0—10 μ g of inhibitor or 0—20 μ l of the solution to be tested in 0.4 ml of 0.02 M sodium glycerophosphate buffer/0.001 M CaCl₂, pH 6.9, and the mixture is equilibrated for 10—20 minutes in a water bath at 35°C. The mixture is then incubated for 5 minutes at 35°C with 0.5 ml of a 1% strength starch solution which has been pre-warmed to 35°C (soluble starch No. 1,252 from Messrs. Merck, Darmstadt), and thereafter 1 ml of dinitrosalicylic acid reagent (according to P. Bernfield in Colowick-Kaplan, Meth. Enzymol., Volume 1, page 149) is added. To develop the colour, the batch is heated for 5 minutes on a boiling water bath and then cooled, and 10 ml of distilled water are added. The extinction at 540 nm is measured against a correspondingly made-up blank without amylase. For evaluation, the amylase activity which is still effective after addition of inhibitor is read off a previously recorded amylase calibration curve and the percentage inhibition of the amylase employed is calculated therefrom. The percentage inhibition is plotted as a function of the quotient

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$$\frac{\mu\text{g of inhibitor} +}{\text{AU} ++}$$

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+ relative to solid
++ AU in non-inhibited batch of the same series
and the 50% inhibition point is read off the curve and converted to AIU/mg of inhibitor.

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Saccharase test

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One saccharase inhibitor unit (SIU) is defined as the amount of inhibitor which inhibits two saccharase units to the extent of 50%. One saccharase unit (SU) is the amount of enzyme which under the test conditions specified splits 1 μ mol of sucrose to glucose and fructose per minute. The μ moles of glucose formed are determined quantitatively by means of the glucose oxidase reaction under conditions under which a further splitting of the sucrose by the saccharase no longer takes place. To carry out the test, 0.05 ml of a saccharase solution¹⁾ adjusted to 0.12 SU is mixed with 0—20 μ g of inhibitor or 0—20 μ l of the solution to be tested and made up to 0.1 ml of 0.1 M sodium maleate buffer of pH 6.0. The mixture is equilibrated for 10 minutes at 35°C and 0.1 ml of an 0.05 M sucrose solution in 0.1 M sodium maleate buffer of pH 6.0, pre-warmed to 35°C, is then added. The mixture is incubated for 20 minutes at 35°C, the saccharase reaction is stopped by addition of 1 ml of glucose oxidase reagent²⁾, and the incubation is

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¹⁾ Solubilised saccharase from the mucous membrane of the small intestine of the pig, according to B. Borgström, A. Dahlquist, Acta Chem. Scand. 12, (1958), pages 1,997, diluted with 0.1 M sodium maleate buffer of pH 6.0 to the appropriate SU content.

²⁾ The glucose oxidase reagent is prepared by dissolving 2 mg of glucose oxidase (Messrs. Boehringer, No. 15,423) in 100 ml of 0.565 M tris-HCl buffer of pH 7.0 and subsequently adding 1 ml of detergent solution (2 g of Triton X 100 + 8 g of 95% strength analytical grade ethanol), 1 ml of dianisidine solution (260 mg of o-dianisidine.2HCl in 20 ml of H₂O) and 0.5 ml of 0.1% strength aqueous peroxidase solution (Messrs. Boehringer, No. 15,302).

continued for a further 30 minutes at 35°C. Thereafter, 1 ml of 50% strength H_2SO_4 is added and a measurement carried out at 545 nm against a corresponding blank. To evaluate the results, the percentage inhibition of the saccharase employed is calculated and converted to SIU/g or SIU/litre from a knowledge of the 50% inhibition point, and using a glucose calibration curve.

Maltase test

One maltase inhibitor unit (MIU) is defined as the amount of inhibitor which inhibits two maltase units to the extent of 50%. One maltase unit (MU) is the amount of enzyme which in one minute, under the test conditions specified below, splits 1 μ mol of maltose into 2 μ mol of glucose. The μ mol of glucose formed are determined quantitatively by means of the glucose oxidase reaction under conditions under which a further splitting of maltose by the maltase no longer takes place. To carry out the test, 0.05 ml of a maltase solution¹⁾ adjusted to 0.060–0.070 MU is mixed with 0–20 μ g of inhibitor of 0–20 μ l of the solution to be tested and made up to 0.1 ml with 0.1 M sodium maleate buffer of pH 6.0. The mixture is equilibrated for 10 minutes at 35°C and 0.1 ml of an 0.05 M maltose solution in 0.1 M sodium maleate buffer of pH 6.0, pre-warmed to 35°C, is then added. The mixture is incubated for 20 minutes at 35°C and the maltase reaction is stopped by addition of 1 ml of glucose oxidase reagent²⁾, and the incubation is continued for a further 30 minutes at 35°C. Thereafter, 1 ml of 50% strength H_2SO_4 is added and a measurement carried out at 545 nm against a corresponding blank.

To evaluate the results, the percentage inhibition of the maltase employed is calculated and converted to MIU/g or MIU/litre from a knowledge of the 50% inhibition point, and using a glucose calibration curve.

The results of the *in vitro* enzyme inhibition test for specific individual homologues and discrete ranges of homologues of the compounds of the invention in which R consists of glucose residues are summarised in the table which follows:

n = number of glucose units per molecule	AIU/g	SIU/g	MIU/g
n = 1	300,000	30,000	5,000
n = 2	300,000	68,000	15,000
n = 3	1,400,000	21,000	5,000
n = 4–6	17,500,000	8,500	—
n = 5–7	30,000,000	2,500	—

As can be seen from the table, the specific inhibitory activity towards pancreas- α -amylase increases greatly with increasing molecular weight in the homologous series; thus, the compounds in which $n = 5–7$ show a 100-fold greater enzyme inhibition *in vitro* than does the compound with $n = 1$ or $n = 2$. On the other hand, the saccharase inhibition is most pronounced with the derivative with $n = 2$, the derivative with $n = 1$ only inhibits half as strongly, and the higher derivatives show a further decrease in the specific activity of saccharase inhibition.

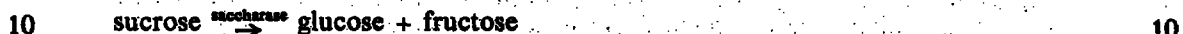
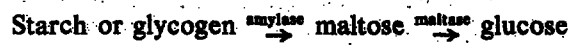
In vivo, the activity in the sucrose overfeeding test runs approximately parallel to the specific inhibitory activity found *in vitro*. On the other hand, in the starch feeding test *in vivo*, we found that the compounds with $n = 1, 2$ and 3 had an

¹⁾ Solubilised maltase from the mucous membrane of the small intestine of the pig, according to B. Borgström, A. Dahlquist, Acta Chem. Scand. 12, (1958), page 1,997, diluted with 0.1 M sodium maleate buffer of pH 6.0 to the appropriate MU content.

²⁾ Preparation of the glucose oxidase reagent: as described for the saccharase test, page 19, footnote 2).

activity increased 10 to 40-fold in comparison to the amylase inhibition *in vitro* (compare Example 18, Table 1 with Tables 3, 4 and 5).

5 It is known that in animals and man hyperglycaemias occur after ingestion of foodstuffs and beverages containing carbohydrates (for example cereal starch, potato starch, fruit, fruit juice, beer or chocolate), these hyperglycaemias being due to a rapid degradation of the carbohydrates by glycoside-hydrolases (for example salivary and pancreatic amylases, maltases and saccharases) in accordance with the following equation:



15 These hyperglycaemias are particularly strongly pronounced and long-lasting in the case of diabetics. With adipose subjects, alimentary hyperglycaemia frequently leads to a particularly powerful secretion of insulin which in turn leads to increased fat synthesis and reduced fat degradation. Following such hyperglycaemias, a hypoglycaemia frequently occurs, due to the insulin secretion, both in metabolically sound and in adipose persons. It is known that both hypoglycaemias and chyme remaining in the stomach promote the production of gastric juice which in turn initiates or favours the development of gastritis or of gastric or duodenal ulcers.

20 It has now been found that the inhibitors of glycoside-hydrolases, according to the invention, which have been obtained and isolated in accordance with the above methods, substantially reduce alimentary hyperglycaemia, hyperinsulinaemia and hypoglycaemia after feeding rats and/or humans with wheat starch or sucrose or maltose and accelerate the passage of the said carbohydrates through the stomach. In addition, they inhibit the resorption of glucose from the intestine. Furthermore, the conversion of carbohydrates into lipids of the fatty tissue and the incorporation of alimentary fat into the fatty tissue depots is reduced or delayed.

25 It is also known that carbohydrates, especially sucrose, are split by micro-organisms in the mouth cavity and that this encourages caries formation.

30 Inhibitors of the glycoside-hydrolases are therefore suitable for use as therapeutic agents for the following conditions: adiposity, hyperlipaemia (atherosclerosis), diabetes, prediabetes, gastritis, gastric ulcers, duodenal ulcers and caries.

35 As stated above, the invention therefore also relates to the use in human and veterinary medicine of the compounds of the invention.

40 The present invention provides a pharmaceutical composition containing as active ingredient a compound of the invention in admixture with a solid or liquefied gaseous diluent, or in admixture with a liquid diluent other than a solvent of a molecular weight less than 200 (preferably less than 350) except in the presence of a surface active agent.

45 The invention further provides a pharmaceutical composition containing as active ingredient a compound of the invention in the form of a sterile or isotonic aqueous solution.

50 The invention further provides a medicated foodstuff or beverage comprising a carbohydrate-containing nutritious material and a compound according to the invention.

55 The invention also provides a medicament in dosage unit form comprises a compound of the invention either alone or in admixture with a diluent.

60 The invention also provides a medicament in the form of tablets (including lozenges and granules), dragees, capsules, pills, ampoules or suppositories comprising a compound of the invention either alone or in admixture with the diluent.

"Medicament" as used in this Specification means physically discrete coherent portions suitable for medical administration. "Medicament in dosage unit form" as used in this Specification means physically discrete coherent portions suitable for medical administration each containing a daily dose or a multiple (up to four times) or sub-multiple (down to a fortieth) of a daily dose of the compound of the invention. Whether the medicament contains a daily dose or, for example, a half, a third, or a quarter of a daily dose will depend on whether the medicament is to be administered once or, for example, twice, three times or four times a day respectively.

The pharmaceutical compositions according to the invention may, for example, take the form of gels, pastes, suspensions, solutions and emulsions of the active ingredient in aqueous or non-aqueous diluents, syrups, granules or powders.

The diluents to be used in pharmaceutical compositions (e.g. granulates) adapted to be formed into tablets, dragees, capsules and pills include the following:

(a) fillers and extenders, e.g. starch, sugars, mannitol, and silicic acid; (b) binding agents, e.g. carboxymethyl cellulose and other cellulose derivatives, alginates, gelatine and polyvinyl pyrrolidone; (c) moisturizing agents, e.g. glycerol; (d) disintegrating agents, e.g. agar-agar, calcium carbonate and sodium bicarbonate; (e) agents for retarding dissolution e.g. paraffin; (f) resorption accelerators, e.g. quaternary ammonium compounds; (g) surface active agents, e.g. cetyl alcohol, glycerol monostearate; (h) adsorptive carriers, e.g. kaolin and bentonite; (i) lubricants, e.g. talc, calcium and magnesium stearate and solid polyethylene glycols.

The tablets, dragees, capsules and pills formed from the pharmaceutical compositions of the invention can have the customary coatings, envelopes and protective matrices, which may contain opacifiers. They can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes and protective matrices may be made, for example, of polymeric substances or waxes.

The ingredient can also be made up in microencapsulated form together with one or several of the above-mentioned diluents.

The diluents to be used in pharmaceutical compositions adapted to be formed into suppositories can, for example, be the usual water-soluble or water-insoluble diluents, such as polyethylene glycols and fats (e.g. cocoa oil and high esters [e.g. C_{14} -alcohol with C_{16} -fatty acid]) or mixtures of these diluents.

The pharmaceutical compositions which are pastes and gels can, for example, contain the usual diluents, e.g. animal and vegetable fats, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide or mixtures of these substances.

The pharmaceutical compositions which are powders can, for example, contain the usual diluents, e.g. lactose, talc, silicic acid, aluminium hydroxide, calcium silicate, and polyamide powder or mixtures of these substances.

The pharmaceutical compositions which are solutions and emulsions can, for example, contain the customary diluents (with, of course, the above-mentioned exclusion of solvents having a molecular weight below 200 except in the presence of a surface-active agent), such as solvents, dissolving agents and emulsifiers; specific examples of such diluents are water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils [for example ground nut oil], glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitol or mixtures thereof.

For parenteral administration, the solutions and emulsions should be sterile, and, if appropriate, blood-isotonic.

The pharmaceutical compositions which are suspensions can contain the usual diluents, such as liquid diluents, e.g. water, ethyl alcohol, propylene glycol, surface-active agents (e.g. ethoxylated isostearyl alcohols, polyoxyethylene sorbitate and sorbitane esters), microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar and tragacanth or mixture thereof.

All the pharmaceutical compositions according to the invention can also contain colouring agents and preservatives as well as perfumes and flavouring additions (e.g. peppermint oil and eucalyptus oil) and sweetening agents (e.g. saccharin).

The pharmaceutical compositions according to the invention preferably contain about 0.1 to 99.5, more preferably from about 0.5 to 95% of the active ingredient by weight of the total composition.

In addition to a compound of the invention, the pharmaceutical compositions and medicaments according to the invention can also contain other pharmaceutically active compounds, especially other orally-administrable antidiabetic agents; examples of these are β -cytropic sulphonylurea derivatives and blood-sugar level-depressing biguanides. The compositions may also contain a plurality of compounds of the invention.

As examples of medicated foodstuffs according to the invention there may be mentioned bread, potato products, fruit juice, beer, chocolate and other

confectionery, and preserves such as jam, to which an effective amount of at least one compound of the invention has been added.

Any diluent in the medicaments of the present invention may be any of those mentioned above in relation to the pharmaceutical compositions of the present invention. Such medicaments may include solvents of molecular weight less than 200 as sole diluent.

The discrete coherent portions constituting the medicament according to the invention (whether in dosage unit form or not) may be, for example, any of the following: tablets, (including lozenges and granules), pills, dragees, capsules, suppositories and ampoules. Some of these forms may be made up for delayed release of the active ingredient. Some, such as capsules, include a protective envelope which renders the portions of the medicament physically discrete and coherent.

The preferred daily dose for administration of the medicaments of the invention is $150-3 \times 10^7$ AIU or $50-1 \times 10^6$ SIU of active ingredient.

The production of the above-mentioned pharmaceutical compositions and medicaments is carried out by any method known in the art, for example, by mixing the active ingredient(s) with the diluent(s) to form a pharmaceutical composition (e.g. a granulate) and then forming the composition into the medicament (e.g. tablets).

This invention further provides a method of combating (including prevention, relief and cure of) the above-mentioned diseases in non-human animals, which comprises administering to the animals a compound of the invention alone or in admixture with a diluent or in the form of a medicament according to the invention.

It is envisaged that these active compounds will be administered perorally, parenterally (for example intramuscularly, intraperitoneally or intravenously), or rectally preferably perorally. Preferred pharmaceutical compositions and medicaments are therefore those adapted for peroral administration, such as suspensions, solutions, pills, tablets, capsules and powder. Administration in the method of the invention is preferably peroral.

In general it has proved advantageous to administer amounts of from $30-3 \times 10^5$ AIU/kg. or $10-1 \times 10^4$ SIU/kg. of body weight per day to achieve effective results. Nevertheless, it can at times be necessary to deviate from those dosage rates, and in particular to do so as a function of the nature and body weight of the human or animal subject to be treated, the individual reaction of this subject to the treatment, the type of formulation in which the active ingredient is administered and the mode in which the administration is carried out, and the point in the progress of the disease or interval at which it is to be administered. Thus it may in some case suffice to use less than the above-mentioned minimum dosage rate, whilst other cases the upper limit mentioned must be exceeded to achieve the desired results. Where larger amounts are administered it can be advisable to divide these into several individual administrations over the course of the day.

The toxicity of the compounds of the invention is very low. The compound prepared in Example 8 (crude preparation containing 26,000 SIU/g.) is tolerated without symptoms, as are the compounds of the invention described in the other Examples, at a dosage of 340,000 SIU/kg. body weight given perorally to mice and rats. Even on intravenous administration, mice tolerated 10,000 SIU/kg. body weight without symptoms.

Explanation of some of the reagents and auxiliaries that can be used in the process of the invention and/or are mentioned above and in the following Examples:—

As ion exchangers, for example the following commercially available products can be used: Amberlite IRA 410 Cl^- (anion exchanger); Amberlite IRC 120 (H^+ form) (strongly acid ion exchanger); Amberlite (HCO_3^- -form) (anion exchanger); Amberlite IRA 410 OH^- (strongly basic ion exchanger); Amberlite IRC 50 H^+ (weakly acid cation exchanger) (the word "Amberlite" is a Registered Trade Mark of the Rohm & Haas Company) and Dowex 50 WX 4H^+ (strongly acid ion exchanger) (the word "Dowex" is a Registered Trade Mark of the Dow Chemical Co., Midland, Michigan, USA).

As cellulose-based anion exchangers, for DEAE-celluloses from Messrs. Schleicher & Schüll can, for example, be used.

As polyacrylamide gels, Biogel-P-2 (Trade Mark of Messrs. Bio-Rad Laboratories, Richmond, California, USA) can, for example, be used.

Maltzin is a natural malt extract from Messrs. Diamalt A.G., Munich (Federal Republic of Germany).

Lewapol is an unspecific adsorption resin from Messrs. Bayer A.G., Leverkusen (Federal Republic of Germany).

Clarcel is a filtering auxiliary from Messrs. CECA, Velizy-Villacoublay, France (the word "Clarcel" is a Registered Trade Mark).

The following Examples 1 to 17 and 19 illustrate the production, in the process of the invention, of typical compounds of the invention. Percentages are by weight unless otherwise indicated.

In the Examples the individual compounds of the invention and their higher molecular weight homologues are identified by reference to the number (n) of monosaccharide units per molecule. In all the Examples, the monosaccharide units are glucose residues.

Example 1.

A glass fermenter filled with 8 litres of nutrient solution containing 5.0% of starch, 1.0% of yeast extract and 0.2% of K_2HPO_4 is inoculated with a 3 day old shaken flask culture of the strain SE 50/13 (CBS 614.71) and the mixture is incubated with intensive stirring and aeration for 3 days at 28°C, giving a culture broth containing 105,000 AIU/ml.

6 litres of this culture broth are cooled to 20°C, the pH is adjusted to 2.5 with half-concentrated HNO_3 , 30 g of Carboraffin active charcoal are added and the mixture is stirred for 10 minutes. It is then centrifuged at 10,000 rpm for 15 minutes and the clear light yellow supernatant liquid is neutralised with NH_3 and then concentrated to 500 ml. The 500 ml of concentrate were stirred for 45 minutes with 200 g of Amberlite IRA 410 Cl^- , the latter was filtered off and the filtrate was treated with 4/5 of its volume (= 400 ml) of methanol in order to precipitate the bulk of the higher-molecular starch degradation products (together with active charcoal residues still present). The mixture is centrifuged for 5 minutes at 5,000 rpm. The 850 ml of supernatant liquid are added dropwise to 4 litres of dry spirit, with intensive stirring. The white flocculent precipitate is filtered off, washed 3 times with dry spirit and twice with ether and dried *in vacuo* at 50°C. Yield 36 g of a white powder containing 10×10^6 AIU/g. This preparation is referred to below in several of the following Examples.

The assess the end products of fermentation and the composition of the final preparation by means of thin layer chromatography, 1 μ l of the fermentation broths or 1 μ g of the preparations is applied to ready-to-use silica gel TLC films (Messrs. Schleicher and Schüll, Dassel, type F 1,500) and the chromatogram is developed twice in n.-butanol/ethanol/water = 50/30/20 (v/v).

To produce a saccharase inhibition colouration, the developed and well-dried plate is sprayed with enzyme gel; (20 ml/20 x 20 cm plate) and the gel is allowed to solidify. The system is then pre-incubated for 5 minutes in a moist chamber at room temperature and then generously sprayed with substrate gel. After this 2nd gel layer has solidified, the plate is introduced into a moist chamber and incubated at 40°C. The inhibition colouration (light spots, red-brown background) develops in 60—90'. At the point in time of optimum colour development, the treatment is discontinued and the plate, with the agar layers thereon, is dried using a warm air blower.

Preparation of the gels:

Enzyme gel: 1.5 g of agarose (from L'Industrie Biologique Francaise) is suspended in 100 ml of 0.2 M Na maleate buffer of pH 6.0 and then dissolved by boiling up. The clear agarose solution is cooled to 50°C and 250 μ l of Triton X—100 solution (2 g of Triton (Registered Trade Mark) X—100 + 8 g of analytical grade ethanol) and 0.5 ml of dianisidine solution (20 mg of dianisidine/1 ml of acetone) are added, with swirling. Directly before using the gel, 1 ml of GOD/POD reagent (12.5 mg of glucose oxidase, degree of purity I, Messrs. Boehringer, order No. 15,423 and 2.5 mg of peroxidase, degree of purity II, Messrs. Boehringer, order No. 15,302, dissolved in 5 ml of maleate buffer) and 4—5 saccharase units from the small intestine of the pig¹⁾ are added. The gel must be kept at 50°C until it is sprayed, since otherwise it solidifies in the nozzles during the spraying process.

¹⁾ Enzyme preparation as described above under "Saccharase Test", footnote 1)

Substrate gel: 0.5 g of agarose is suspended in 100 ml of Na maleate buffer of pH 6.0 and dissolved whilst boiling. The solution is then cooled to 50°C and 100 μ l of Triton (2 g of Triton X-100 + 8 g of analytical grade ethanol) are then added, followed by 1 g of sucrose (Serva No. 35,579). After the sucrose has dissolved, the gel is ready to use.

For the amylase inhibition colouration, the developed and dried thin layer chromatography plate is sprayed with an amylase gel (20 ml/20 x 20 cm plate) and is allowed to solidify. After 5 minutes of pre-incubation at room temperature, the plate bearing the gel layer is introduced into an 0.5% strength starch solution (1 g of starch, Merck No. 1,252, dissolved, with boiling, in 200 ml of 0.2 M glycerophosphate buffer, 0.01 M CaCl_2 , pH 6.9) and is left therein for 2 minutes at 40°C whilst swirling the solution. The plate is then well rinsed with distilled water and dipped into a dilute I_2 solution (4 ml of I_2 stock solution per 500 ml of H_2O ; I_2 stock solution: 2.2 g of I_2 + 4.4 g of KI dissolved in 100 ml of H_2O) in order to colour the starch which has not been degraded. After about 1 minute, the colouration is at an optimum. It is photographed immediately since the blue spots fade rapidly.

Preparation of the amylase gel:

1 g of agarose is dissolved in 100 ml of 0.2 M sodium glycerophosphate/0.01 M CaCl_2 buffer of pH 6.9 at 100°C and after cooling to 50°C, 100 μ l of Triton X-100 (2 g of Triton X-100 + 8 g of analytical grade ethanol) are added. Directly before spraying, 100 μ l of an amylase crystal suspension (10 mg of pig's pancreas amylase/ml of saturated NH_4 sulphate solution, Messrs. Boehringer, No. 15,017) are added.

Example 2.

If a 1 litre Erlenmeyer flask containing 120 ml of a nutrient solution consisting of 4% of starch, 2.4% of glucose, 0.9% of casein hydrolysate and 0.9% of yeast extract, pH adjusted to 7.6 with NaOH, mixed with 0.4% of CaCO_3 and sterilised for 30 minutes at 121°C, is inoculated with 3 ml of a pre-culture of the strain SE 82 (CBS 615.71), grown in a nutrient solution consisting of 2% of starch, 1% of glucose, 0.5% of casein hydrolysate and 1% of yeast extract, pH adjusted to 7.2 with NaOH, treated with 0.4% of CaCO_3 and sterilised for 30 minutes at 121°C, and the whole is incubated for 5 days at 28°C on a rotary shaking machine, a culture solution containing 122,000 AIU/ml is obtained. For working up, the mycelium is separated from the combined culture solutions by centrifuging at 12,000 rpm, 300 ml of the culture filtrate are brought to pH 2.5 with half-concentrated HNO_3 and the mixture is stirred for 10 minutes with 2.5 g of analytical grade active charcoal. After separating off the charcoal at 12,000 rpm, the solution is neutralised to pH 6 with 10 N KOH, 300 ml of methanol are added, the mixture is allowed to stand briefly and the precipitate is removed at 12,000 rpm. If the supernatant liquid is now added dropwise to 3 litres of ethanol and the precipitate is isolated, after brief standing, by centrifuging at 12,000 rpm and is washed twice with absolute ethanol and once with ether and dried *in vacuo*, 2.23 g of a product containing 7.45×10^6 AIU/g are obtained, which contains more than 95% of homologues of the compounds of the invention with $n > 4$.

Example 3.

If a 1 litre Erlenmeyer flask with 120 ml of a nutrient solution of composition 3.5% of glucose, 2% of starch 0.5% of casein hydrolysate, 1.3% of yeast extract, 0.3% of CaCO_3 and 0.3% of K_2HPO_4 , adjusted to pH 7.8 before sterilisation and sterilised for 30 minutes at 121°C, is inoculated with 6 ml of a pre-culture of the strain SE 50/110 (CBS 674.73) in a nutrient solution consisting of 3% of soya flour, 3% of glycerol and 0.2% of CaCO_3 and the mixture is incubated for 3-4 days on a rotary shaking machine at 24°C, a culture solution which contains 153,000 AIU/ml and 12,000 SIU/litre is obtained.

1 litre of culture solution was adjusted to pH 2.5 with HNO_3 and the mixture was stirred for 10 minutes with 5 g of active charcoal and then centrifuged for 30 minutes at 5,000 rpm. It was then neutralised by adding 25 g of Amberlite IRA 410 (OH^- -form). The neutral supernatant liquid was concentrated to 100 ml on a rotary evaporator, mixed with 100 ml of methanol and filtered. The filtrate was stirred into 2 litres of dry spirit and the precipitate which separated out was filtered off, washed 3 times with acetone and ether and dried *in vacuo*.

Yield 14 g of a white powder containing 5×10^6 AIU/g and predominantly containing homologues of the compounds of the invention with $n > 4$.

Example 4.

If the procedure of Example 3 is followed but with the addition of 0.5% starch, a culture broth containing 40,000 AIU and 184 SIU/ml is obtained after 4 days' fermentation. The culture broth contains compounds of the invention and homologues thereof with $n = 1$ upwards.

Example 5.

If a 1 litre Erlenmeyer flask which contains 120 ml of nutrient solution of composition 3% of glucose, 0.6% of casein hydrolysate, 1.6% of yeast extract, 0.3% of CaCO_3 and 0.3% of K_2HPO_4 , pH adjusted to 7.8 with KOH before sterilisation, is inoculated with a pre-culture of the strain SE 50/110 (CBS 674.73) according to Example 3 and incubated for 4 days at 24°C on a rotary shaking machine, a culture broth of 10,800 SIU/litre, which predominantly contains the compound of the invention with $n = 1$, is obtained.

5 litres of culture filtrate, separated from the mycelium at 13,000 rpm, were adjusted to pH 2.5 with half-concentrated HNO_3 and stirred for 15 minutes with 55 g of active charcoal ("Merck") and 200 g of Clarcel. After removing the solids by suction filtration, the filtrate was neutralised to pH 7 with concentrated ammonia and the solution was concentrated to 1.5 litres and precipitated with a five-fold amount of ethanol. The resulting flocculent precipitate was separated off using a continuous-flow rotor at 12,000 rpm and the yellowish supernatant liquid was concentrated to 150 ml and centrifuged at low speed to separate off minor proportions of undissolved material. 50 ml of this solution were charged onto a column filled with Amberlite IR-120 (H^+ form) (30×300 mm; 30 ml of H_2O per hour). After a total of 300 ml of eluate, which contains inert saccharides and a proportion of non-adsorbed components having an inhibiting action, had been collected, the exchanger was transferred into a beaker with about 400 ml of H_2O and concentrated ammonia was added, whilst stirring, until the pH had reached a value of 11.5. After stirring for a further 30 minutes, the exchanger was separated off, the liquid was concentrated to 1/20 of its volume and filtered through a column (20×150 mm) containing Amberlite IRA-410 (HCO_3^- form) and about 500 ml of eluate were collected at a flow speed of 30 ml/hour; the eluate was concentrated and after lyophilisation gave 1.3 g of crude product.

For further purification, the crude product was fractionated on Bio-Gel P-2, 100-200 mesh (Messrs. Bio-Rad, Munich). A column of 50 mm diameter and 450 mm length was used for this purpose and was operated with H_2O at a flow speed of 40 ml per hour, fractions of 10 ml each being collected. All fractions were tested by means of the anthrone test for carbohydrates and by means of the saccharase inhibition test for components having an inhibiting action. The fractions containing saccharase inhibitor were further examined by thin layer chromatography, in accordance with Example 1, for their content of individual components. The fractions which contained the compound of the invention with $n = 1$ were combined, concentrated and lyophilised. 35 mg of the compound with $n = 1$, of 0.3×10^6 AIU/g and 30,000 SIU/g, were obtained.

Example 6.

If 1 l Erlenmeyer flasks each containing 120 ml of a nutrient solution of composition 5% of starch, 1% of yeast extract and 0.2% of K_2HPO_4 are each inoculated with 2 ml of a pre-culture according to Example 3 and incubated for 3 days at 28°C , culture solutions with the following yield of amylase inhibitor are obtained:

Strain	AIU/ml
SE 50 (CBS 961.70)	37,000
SE 50/13 (CBS 614.71)	109,000
SE 50/110 (CBS 674.73)	53,500

The inhibitor consists predominantly of compounds of the invention with $n = 4$, in admixture with higher homologues thereof.

Example 7.

If 1 l Erlenmeyer flasks each containing 120 ml of nutrient solution of composition 1.3% of maltose, 3.5% of glucose, 0.5% of casein hydrolysate, 1.3% of yeast extract, 0.3% of CaCO_3 and 0.3% of K_2HPO_4 are each inoculated with 2 ml of a pre-culture according to Example 3, the following yields are obtained after 4 days' incubation with various strains on rotary shaking machines at 24°C.

Strain	SIU/ml	AIU/ml
SE 50 (CBS 961.70)	25	580
SE 50/13 (CBS 614.71)	14.8	1,460
SE 50/110 (CBS 674.73)	57.9	755

The products consist predominantly of compounds of the invention with $n \geq 4$.

Example 8.

If a fermenter containing 100 l of nutrient solution of composition 3.5% of glucose, 2.5% of dry powdered malt extract, 0.5% of casein hydrolysate, 1.3% of yeast extract, 0.3% of CaCO_3 , 0.3% of K_2HPO_4 and 0.1% of anti-foaming agent is inoculated with 5 l of a pre-culture according to Example 3 and incubated for 5 days at 24°C with stirring and aeration, a culture solution of 73,000 SIU/l is obtained, which predominantly contains the compound of the invention with $n = 2$.

A 90 litre fermentation batch together with the mycelium is adjusted to pH 2.5 on a pH meter by means of concentrated HNO_3 and 900 g (= 1%) of active charcoal (Merck) are added whilst stirring in order to adsorb the bulk of the dyestuffs formed. The mixture is stirred for 15 minutes, the mycelium and the bulk of the charcoal were separated off on a centrifuge at 3,000 rpm and the supernatant liquid, with addition of 3 kg of Clarcel, is finally filtered through a pressure filter. 65 l of yellow-brown, clear filtrate of SIU content 60,000 SIU/litre are obtained.

The filtrate is adjusted to pH 7 with concentrated NH_3 and stirred with 1,300 g (2%) of active charcoal (Merck) for 30 minutes in order to adsorb the active substance. The mixture is filtered through a pressure filter and the active charcoal sediment was washed 3 times with 10 litres of distilled water. The charcoal is then thoroughly pressed dry and stirred with 3 times 4 litres of 50% strength acetone at pH 2.5, in each case for 15 minutes, so as to desorb the active substance from the charcoal. The acetone desorbates are combined after removing the charcoal by filtration. The combined desorbate is concentrated to 250 ml on a rotary evaporator, an equal volume (250 ml) of methanol is added and the mixture is filtered through a folded filter. The filtrate (480 ml) is added dropwise to 5 litres of acetone, with vigorous stirring. The precipitate which separated out is filtered off and washed 3 times with acetone and ether. It is then dried *in vacuo* at 35°C. Yield 230 g of crude product containing 8,500 SIU/g.

25 g of the above crude product are dissolved in 1 litre of H_2O and stirred with 300 g of Dowex 50 WX 4 H^+ (200—400 mesh) for 30 minutes. The resin is filtered off and rinsed 3 times with 2 litres of 0.001 N HCl . The washed Dowex is then suspended in 500 ml of H_2O and the suspension adjusted to pH 9.0 on a pH meter by addition of 25% strength NH_3 . Thereafter 2 further desorptions are carried out, each with 500 ml of 0.6% strength NH_3 and the desorbates are combined and concentrated to 100 ml on a rotary evaporator. To decolourise this concentrate, it is stirred for 5 minutes with 2 g of DEAE-cellulose (Messrs. Schleicher and Schüll, No. 02035, 0.6 milliequivalent/g), and then centrifuged. The light yellow supernatant liquid is mixed with an equal volume (100 ml) of methanol and the mixture is then added dropwise to 2 litres of acetone, with intensive stirring. The precipitate is filtered off, washed with acetone and ether and dried *in vacuo* at 35°C. Yield, 4.2 g containing 26,000 SIU/g. For additional fine purification, the 4.0 g of inhibitor are gel-filtered, in 0.5 g portions, through Biogel P—2. For this purpose, each 0.5 g of the preparation is dissolved in 10 ml of H_2O and the solution was charged onto a Biogel P—2 column (200—400 mesh, Messrs. Bio-Rad) of 5 cm diameter and 95 cm length. The column is developed in water at a flow rate of 80 ml/hour. 12 ml fractions are collected. For all fractions, the total carbohydrate content (in the form of the anthrone test, as an extinction at E_{620}) and the content

of saccharase inhibitor and amylase inhibitor is determined. In addition, the fractions are tested by thin layer chromatography (enzyme inhibition colouration according to Example 1).

The fractions in which the compounds with $n = 4-6$ were detected are combined, concentrated to 10 ml *in vacuo* and precipitated by dropwise addition to 200 ml of dry spirit. The precipitate is centrifuged off, washed with acetone and ether and dried *in vacuo*; yield from 4.0 g of crude inhibitor: 0.2 g of compounds of the invention with $n = 4-6$ and containing 17.5×10^6 AIU/g and 8,500 SIU/g. The fractions containing the compound with $n = 3$ are worked up in the same manner, the precipitation being carried out with 200 ml of acetone; yield from 4.0 g of crude inhibitor: 0.1 g of compound of the invention with $n = 3$, containing 1.4×10^6 AIU/g and 21,000 SIU/g. 0.9 g of the compound of the invention with $n = 2$, containing 0.3×10^6 AIU/g and 68,000 SIU/g is isolated from the fractions (precipitation with acetone) containing the compound with $n = 2$.

Example 9.

If 3 small fermenters each containing 8 l of a nutrient solution with 7.5% of dry powdered malt extract, 0.3% of casein hydrolysate, 0.7% of yeast extract, 0.3% of CaCO_3 and 0.3% of K_2HPO_4 are inoculated with 5% of a preculture of the strain SE 50/110 (CBS 674.73) (obtained according to Example 3), 5 days' incubation at 24°C gives a culture broth of 73 SIU/ml which predominantly contains compounds of the invention with $n = 2$. After centrifuging (30', 3,000 rpm) to separate off the mycelium, 20.5 litres of a deep brown culture solution containing 67,000 SIU/l were obtained. This solution is adjusted to pH 3.5 with HNO_3 and 60 g of Lewapol (Ca 9221, 0.35 mm particle size, Messrs. Bayer)/l = 1.23 kg of Lewapol were added to decolourise the solution. After stirring for 20 minutes, the mixture is filtered using a Seitz K 3 filter. The decolourised culture solution is neutralised with NH_3 (18.5 l, 67,000 SIU/l), 20 g of active charcoal/l = 370 g are then stirred in to adsorb the active substance, and the mixture was stirred for 30 minutes. It is then filtered through a K 3 filter which is covered with a layer of the filter aid Clarcel. The filtrate (17.5 l, 3,600 SIU/l) is discarded. The charcoal residue is washed 3 times with 2 l of distilled H_2O . To desorb the active substance from the charcoal, the latter is stirred 3 times in succession, each time for 15 minutes, with 1 l of 80% strength acetone at a time, the pH being adjusted to 2.5 with concentrated HCl . The desorbates are combined (2.4 l, 371,000 SIU/l). 20 g of Dowex H^+/l (Dowex 50W $\times 4$, H^+ form, produced by Messrs. Serva, Heidelberg equivalent to 46 g of Dowex) were introduced into this desorbate and the mixture was stirred for 20 minutes. The resin is then filtered off (Dowex fraction I) and rinsed with a little 75% strength acetone. The filtrate and wash liquid (3 l = 215,000 SIU/l) are stirred with 60 g of Amberlite IRA 410 (OH^- form) (Messrs. Serva, Heidelberg)/l until pH 7 was reached. The mixture is then filtered and the filtrate (2.8 l, 219,000 SIU/l) is mixed with 72 g of Dowex H^+ and stirred for 20 minutes. Whilst doing so, the pH is kept at 3.0 by hanging a porous nylon pouch, filled with Amberlite 410 OH^- , into the mixture. The Dowex is then filtered off (Dowex fraction II) and the filtrate (2.6 l, 27,000 SIU/l) is discarded.

The Dowex fractions I and II are each washed individually 3 times with 75% strength acetone at pH 3.5 and then each desorbed 3 times with 100 ml of 0.6% strength NH_3 at a time (Dowex fraction I) or with 150 ml of 0.6% strength NH_3 at a time (Dowex fraction II). During the first desorption, during which the amount of ammonia does not suffice to neutralise the Dowex resin, the pH is adjusted to 9 on a pH meter by addition of concentrated NH_3 . The three desorbates from Dowex fraction I and II are respectively combined, concentrated almost to dryness on a rotary evaporator, taken up in 50 ml of H_2O , adjusted to pH 3-4 on a pH meter by means of HCl , and mixed with 50 ml of methanol. The solutions are added dropwise to 1.5 l of absolute acetone, whilst stirring, and the precipitate formed is filtered off and washed 3 times with acetone and once with ether. It is dried *in vacuo*.

Yield: Fraction I 6.5 g 25,000 SIU/g
Fraction II 12.3 g 36,000 SIU/g

Fraction I and II mainly contain, as the inhibiting constituents, the compound of the invention with $n = 2$, in addition to small proportions of the next-higher homologue ($n = 3$).

The following Table shows the saccharase-inhibiting activities of the preparation at successive stages.

Yield	Volume (l)	SIU/l	Total SIU	Yield of SIU %
1) Culture solution	20.5	67,000	1,373,500	100
2) After discolouration with Lewapol	19.5	67,000	1,306,500	95
3) After active charcoal adsorption	18.5	3,600	66,600	(4.8 discarded)
4) 1st desorbate	0.7	742,000	519,400	37.8
5) 2nd "	0.9	329,000	296,100	21.6
6) 3rd "	0.8	85,000	68,000	5.0
			883,500	64.4
7) Mixed desorbate (4-6)	2.4	371,000	890,400	64.8
8) After 1st Dowex adsorption	3.0	215,000	645,000	
9) After neutralisation with IRA OH ⁻	2.8	219,000	613,200	
10) After 2nd Dowex adsorption	2.5	27,000	67,500	(4.9 discarded)
11) Combined NH ₃ desorbates of Dowex fraction I	0.29	682,000	197,780	14.4
12) Combined NH ₃ desorbates of Dowex fraction II	0.45	1,419,000	638,550	46.5
				60.2
13) Precipitate, Fraction I	6.5 g	25,000/g	162,500	11.8
Precipitate, Fraction II	12.3 g	36,000/g	442,800	32.2
				44.0

Example 10.

200 g of a preparation as described in Example 1 were dissolved in 940 ml of distilled water and 60 ml of concentrated H_2SO_4 and the mixture was warmed under reflux for 4 hours (internal temperature: 98–100°C; oil bath temperature: 140°C). 10 g of active charcoal (Merck Art. 2186) were added to the cooled black-brown solution and the mixture was stirred for 1 hour. The active charcoal was then filtered off and washed with water and the filtrate was adjusted to pH = 7 to 8 with about 250 ml of 10 N KOH. The solution was stirred for 1 hour with 50 g of active charcoal. The charcoal was filtered off and washed with 2 l of water and the filtrate was discarded. For desorption, the charcoal was digested overnight with 2 l of 30% strength alcohol. Finally, the charcoal was filtered off and the alcoholic solution was concentrated on a rotary evaporator. Residue: 6.2 g. This crude product (6.2 g) was dissolved in 500 ml of water and the solution was gently stirred with 30 g of Amberlite IR 120 (H⁺ form) for 1 hour. The exchanger was filtered off and washed with distilled water until the filtrate was neutral and free from glucose. The exchanger was then stirred overnight with 15 ml of 25% strength NH_3 in 1,000 ml of H_2O , separated off and discarded. The filtrate was concentrated on a rotary evaporator.

Residue: 3.7 g.

For further purification, a chromatography on cellulose was carried out. 4.5 g of the material desorbed from the exchanger were applied to a 1 m long and 2.5 cm wide column filled with cellulose. The running agent used was initially 5:1 ethanol/ H_2O , and 3:1 ethanol/ H_2O was initially used to eluate the compound of the invention with $n = 1$. Fractions of 14 ml were collected at a drip speed of 20 drops per minute. The individual fractions were examined by thin layer chromatography. Fractions 47–85 gave, after concentration, 1.6 g of a compound of the invention with $n = 1$, exhibiting a pale brownish discolouration. The discolouring impurities were quantitatively insignificant. The compound with $n = 1$ was obtained as a colourless resin if the purification step with a strongly acid ion exchanger was carried out on a column and not by the batch process.

Example 11.

200 g of a preparation as described in Example 1 were dissolved in 940 ml of distilled water and 60 ml of concentrated H_2SO_4 and the solution was warmed under reflux for 1/4 hour (internal temperature: 98–100°C; oil bath temperature: 140°C). 10 g of active charcoal (Merck, Art. 2186) were added to the cooled, black-brown solution and the mixture was stirred for 1 hour. The active charcoal was then filtered off and washed with water and the filtrate was adjusted to pH = 7 to 8 with about 250 ml of 10 N KOH. The solution was stirred with 50 g of active charcoal for 1 hour. The charcoal was filtered off and washed with 2 l of water and the filtrate was discarded. For desorption, the charcoal was digested overnight with 2 l of 30% strength alcohol. Finally, the charcoal was filtered off and the alcoholic solution was concentrated on a rotary evaporator.

Residue: 8.0 g.

The residue was taken up in 15 ml of H_2O and applied to a column (height: 20 cm, diameter 2.4 cm) filled with 50 g of Amberlite IR 120 (H⁺ form). The solution was absorbed at 3 drops/minute and the column was rinsed with water (12 drops/minute) until all non-basic constituents had been removed. The basic products were then eluted from the column with 0.5% strength NH_3 (12 drops/minute) and the aqueous solution was evaporated to dryness on a rotary evaporator.

Residue: 4.1 g.

2 g of this residue were dissolved in a little water and applied to a column (height: 200 cm ϕ : 3.0 cm) filled with Sephadex G-15. The column was eluted with water. Fractions of 2 ml each were collected at a flow speed of 8 ml/hour. The individual fractions were examined by thin layer chromatography. Fractions 85–94 gave 280 mg of the compound of the invention with $n = 2$, having a specific activity of 50,000 SIU/g.

Example 12.

If 2 g of a preparation as described in Example 1, in 60 ml of 20 mM sodium glycerophosphate buffer of pH 6.9, containing 1 mM of $CaCl_2$, are incubated with 1 g of α -amylase from *Aspergillus* spec. (SERVA No. 13,418) for 120 hours at 37°C with constant stirring and finally heated to 100°C for 5 minutes, and undissolved matter is centrifuged off at 4,000 rpm, lyophilisation of the solution gives 1.9 g of a product is tested by thin layer chromatography and

saccharase inhibition discolouration as described in Example 1, it is found that the compounds having an inhibiting action which are present are essentially the compounds of the invention with $n = 1, 2$ and 3 .

Example 13.

If 2 g of a preparation as described in Example 1, in 30 ml of 20 mM acetate buffer of pH 4.8, are incubated with 1.25 mg of β -amylase from sweet potato (BOEHRINGER 15,471) for 120 hours at 37°C, with constant stirring and finally heated to 100°C for 5 minutes, and undissolved matter is centrifuged off at 4,000 rpm, lyophilisation of the solution gives 1.5 g of a product with 1,800 SIU/g and 3.8×10^6 AIU/g. If this product is tested by thin layer chromatography and saccharase inhibition discolouration as described in Example 1, it is found that the compounds having an inhibiting action which are present are essentially the compounds of the invention with $n = 2$ and 3 .

Example 14.

If a 200 ml Erlenmeyer flask containing 25 ml of a nutrient solution of composition 0.1% K_2HPO_4 , 0.2% of $(NH_4)_2SO_4$, 0.05% of $MgSO_4$, 0.05% of KCl, 0.01% of $FeSO_4$ and 2% of a preparation as described in Example 1 is inoculated with a spore suspension of the strain *Asp. niger* ATCC 11,394 and incubated at 28°C on a rotary shaking machine, the AIU concentration falls from 210,000 AIU/ml to 53,000 AIU/ml after 6 days and to 21,300 AIU/ml after 10 days. At the same time the SIU/ml content rises from 7.0 to 72 SIU/ml.

20 ml of a solution which has been incubated with the spore suspension for 10 days are centrifuged for 30 minutes at 3,000 rpm to separate off the mycelium. 15 ml of supernatant liquid (72,000 SIU/l) are desalinated by stirring for 30 minutes with 2 g of Amberlite IRC 50 H^+ and 1 g of Amberlite IRA 410 OH^- (conductivity less than 2 mSiemens). The mixture is filtered and the filtrate allowed to run at the rate of 5 ml/hour through a column (1 cm $\times$ 10 cm) of Dowex H^+ equilibrated in 0.001 N HCl. The column is then rinsed with 200 ml of 0.001 N HCl. For desorption, 0.6% strength NH_3 solution is pumped through the column (10 ml/hour) and 5 ml fractions are collected. The fractions containing the saccharase-inhibiting activity are combined, concentrated to 2 ml on a rotary evaporator and mixed with 2 ml of methanol. This solution is adjusted to pH 3–4 and precipitated by adding it dropwise to 100 ml of acetone. The precipitate is filtered off, washed with acetone and ether and dried *in vacuo*. Yield: 26 mg containing 28,000 SIU/g and consisting of compounds of the invention with $n = 2$ and 3 . The isolation of the pure compound of the invention with $n = 2$ from this product is effected as described in Example 8 by gel filtration through a column containing Bio-Gel P—2. 7 mg of the compound of the invention with $n = 2$, of 60,000 SIU/g, are obtained.

Example 15.

2 litres of culture filtrate obtained from a fermentation batch as described in Example 5 by centrifuging off the mycelium at 13,000 rpm and having an activity of 13,000 SIU/g were stirred with 500 g of a mixture of 2.5 parts of Amberlite IRC—50 (H^+ form) and 1 part of Amberlite IRA—410 (OH^- form) for 1 hour in order to reduce the salt content (conductivity of the culture filtrate: about 10 mS). The exchanger was separated off and the solution was concentrated to a little less than 100 ml and centrifuged for 15 minutes at 20,000 rpm to remove undissolved constituents. The supernatant liquid was made up to 100 ml; it now had a conductivity of 3.5 mS and was further purified by applying it to a column (55 $\times$ 400 mm) of P-cellulose (SERVA No. 45,130, pre-treated according to known methods and equilibrated in 5 mM ammonium phosphate buffer, pH 5.5). The abovementioned phosphate buffer served as the running agent; the flow rate was 90 ml/hour and fractions of 18 ml volume were collected.

After the fractions of the eluate had been tested for their carbohydrate content (by means of the anthrone test) and for their content of saccharase-inhibiting components (by means of the saccharase inhibition test), the fractions which in the anthrone test had proved almost free of carbohydrates and equally in the saccharase inhibition test had proved particularly active were combined (fractions 60–170), concentrated to 150 ml and filtered through a column (50 $\times$ 300 mm) containing Amberlite IRA—410 (HCO_3^- form). For better control of the deionisation, the eluate was collected in fractions (10 ml per fraction in 20 minutes) and tested for carbohydrate (by means of the anthrone test: in each case

virtually negative), for phosphonate (by means of ascorbic acid-molybdate reagent: in each case negative) and for saccharase inhibition (by means of the enzyme inhibition test). The fractions having an inhibiting action (3—30) were combined, concentrated, lyophilised, redissolved and lyophilised so as to give 280 mg of crude inhibitor.

For further purification, the crude inhibitor was fractionated on Bio-Gel P—2 as described in Example 5. From the fractions which contained, pure, the compound of the invention with $n = 1$, 30 mg of a product with 0.3×10^6 AIU/g and 35,000 SIU/g were isolated after lyophilisation.

Example 16.

To isolate the compounds of the invention with $n = 5—7$, the starting material used can be, for example, a preparation such as described in Example 1.

For this purpose, 30 g of the preparation according to Example 1 were dissolved in 250 ml of H_2O . The conductivity of the resulting solution was 10 mS and the pH was 5.5. The solution was desalinated by adding 60 g of Amberlite IRC 50 H^+ (weakly acid cation exchanger which only binds traces of the amino-sugar derivatives from aqueous solution) and 20 g of Amberlite IRA 410 OH^- and stirring for 20 minutes. The filtrate (conductivity 0.5 mS, pH 3.5) was adjusted to pH 3.0 with 1 N HCl (conductivity 0.6 mS). This solution was pumped at the rate of 42 ml/hour through a column filled with Dowex 50 W $\times 4$, 200—400 mesh. (H^+) (ϕ 2.5 cm, height 40 cm, equilibrated in 0.001 N HCl) and the Dowex was then rinsed with 2 l of 0.001 N HCl. After washing the column, elution was carried out with 1.2% strength aqueous ammonia and 10 ml fractions were collected. The fractions having an inhibiting action were combined, the ammonia was stripped off *in vacuo* and the solution was then concentrated *in vacuo* to 30 ml. The product was precipitated by dropwise addition to 600 ml of dry spirit and the precipitate was filtered off, washed with alcohol and ether and dried *in vacuo*. Yield 4.4 g, containing 26.5×10^6 AIU/g.

0.5 g portions were subjected to a fine purification by application to a preparative Biogel P—2 column, as described in Example 8, and development. The fractions which according to a thin layer chromatogram (amylase inhibition colouration) contain compounds of the invention with $n = 5—7$ were combined, concentrated *in vacuo* and precipitated with dry spirit as described above. Yield from 0.5 g of crude product: 0.2 g of amino-sugar derivatives with $n = 5—7$, containing 30×10^6 AIU/g and 2,500 SIU/g.

Example 17.

This example illustrates how the compounds of the invention can be eluted from cation exchanger under acid conditions.

A column of 1.5 cm. diameter is filled with 30 g. (wet weight) of Dowex 50 W $\times 4$, (H^+) 200—400 mesh in 0.001 n HCl. Finally 500 ml. of mixed desorbate (400 000 SIU/L), pH 2.5, 60% acetone), obtained according to Example 9 (Table, run No. 7) are pumped through the column in about 1 hour and washed finally with 500 ml. of 0.001 n HCl. Under these conditions only trace activity is eluted. Finally desorption therefrom with 0.0125 n HCl was effected, whereby the conductivity of the column eluate was measured. The SIU-content of the eluate was also tested. The active fractions 74—100 were combined and neutralized by the addition of Amberlite IRA 410 OH^- , then reduced to 5 ml., reacted with 5 ml. of methanol, and precipitated by dropping into 200 ml. acetone. After washing with acetone and ether vacuum-drying was effected.

Yield 1 g. of the compound of the invention with $n = 2$ with 65000 SIU/g.

From the active initial fraction the compounds with $n = 3$ and $n = 4$ could be obtained.

This process of acid desorption therefore makes possible, in contrast to the alkaline desorption, a fractionation of the homologous amino-sugar derivatives.

The following biological tests demonstrate the amylase- and saccharase-inhibiting activity of typical compounds of the invention.

I. Action of glycoside-hydrolase inhibitors and inhibition of glucose resorption in rats and man.

To produce an alimentary hyperglycemia and hyperinsulinaemia fasting rats ($n = 6$) or fasting human subjects are given either 2.5 g of sucrose or 2.5 g of maltose or 1 g of boiled starch or 2.5 g of glucose/kg body weight orally or 50 g of sucrose or 50 g of maltose or 50 g of boiled starch or 50 g of glucose/person, orally,

- 5 in aqueous solution or as a suspension. Six other rats are given the said carbohydrates in the same amount and, in addition, one of the active compounds mentioned in the Examples, in the dosage indicated. Healthy human subjects are given one of the carbohydrates mentioned alternately with and without one of the active compounds mentioned, at intervals of 2 or more days. The blood glucose and the serum insulin are measured before the carbohydrate overfeeding ± active substance, and at short intervals of time from five minutes up to three hours thereafter, in the blood from the retro-orbital venous plexus of rats or in the cubital venous or capillary blood of the test persons. The blood glucose determinations are carried out in the Auto-Analyser (Technicon [®], according to HOFFMAN: J. biol. Chem. 120, 51 (1937), or enzymatically by means of glucose oxidase and *o*-dianisidine hydrochloride) and the serum insulin determinations are carried out according to the method of HALES and RANDLE: Biochem. J. 88, 137 (1963)). 5
- 10 II. Inhibition of the conversion of carbohydrates to fatty tissue lipids. 10
- 15 Six rats are given one of the carbohydrates mentioned in I, in the inactive form, together with a suitable dose of the same carbohydrate which, however, is radioactively (¹⁴C) labelled, with or without an active substance from the above Examples administered by means of a probang. At short intervals of time after the beginning of the experiment, the animals are killed and epidymal and/or perirenal fatty tissue is isolated, the lipids are extracted with suitable solvents of fats, for example chloroform/methanol (2:1) and the metabolites which do not contain lipid are washed out. The radioactivity of the lipids isolated in this way is measured in a liquid scintillation counter. The activity is quoted in dpm/1 g of fatty tissue. 15
- 20 20

TABLE 1 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of starch $\pm$ active substance from Example 16, compounds with n = 5-7 (s = standard deviation; P = probability of error)

	10	15	20	30	45 mins after admini- stration
Control without starch	55 $\pm$ 4.8	63 $\pm$ 5.9	67 $\pm$ 5.8	72 $\pm$ 4.7	66 $\pm$ 5.0
Control with starch	103 $\pm$ 11	117 $\pm$ 8.6	118 $\pm$ 8.5	128 $\pm$ 8.9	109 $\pm$ 11
12,000 AIU + "	95 $\pm$ 4.3	107 $\pm$ 3.3	102 $\pm$ 5.5	111 $\pm$ 3.0	102 $\pm$ 6.7
24,000 AIU + "	87 $\pm$ 8.6	94 $\pm$ 8.8	89 $\pm$ 8.5	98 $\pm$ 8.3	91 $\pm$ 4.3
	74 $\pm$ 8.9	84 $\pm$ 7.2	84 $\pm$ 6.9	85 $\pm$ 6.5	85 $\pm$ 4.9

TABLE 2 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of starch $\pm$ active substance from Example 8, compounds with n = 4-6.

	10	15	20	30	45 mins. after admini- stration
Control without starch	55 $\pm$ 4.8	63 $\pm$ 5.9	67 $\pm$ 5.8	72 $\pm$ 4.7	66 $\pm$ 5.0
Control with starch	103 $\pm$ 11	117 $\pm$ 8.6	118 $\pm$ 8.5	128 $\pm$ 8.9	109 $\pm$ 11
6,000 AIU + starch	99 $\pm$ 4.5	118 $\pm$ 5.8	104 $\pm$ 8.1	123 $\pm$ 11	107 $\pm$ 7.3
12,000 AIU + "	90 $\pm$ 5.2	98 $\pm$ 8.2	99 $\pm$ 4.1	117 $\pm$ 6.7	104 $\pm$ 8.2
24,000 AIU + "	74 $\pm$ 5.5	82 $\pm$ 2.9	83 $\pm$ 3.7	96 $\pm$ 6.6	85 $\pm$ 6.4
----- P < 0.05	P < 0.01	----- P < 0.001 against control with starch			

TABLE 3 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of starch $\pm$ active substance from Example 8, compound of invention with $n = 3$

Time (mins.) after administration:	5	10	15	20	30	45
Control without starch	52 $\pm$ 2.3	75 $\pm$ 7.9	57 $\pm$ 9.6	78 $\pm$ 9.6	82 $\pm$ 6.5	70 $\pm$ 6.9
Control with starch	93 $\pm$ 7.3	133 $\pm$ 12	127 $\pm$ 9.3	152 $\pm$ 15	156 $\pm$ 15	124 $\pm$ 7.7
750 AIU + starch	86 $\pm$ 8.5	107 $\pm$ 5.0	117 $\pm$ 8.7	121 $\pm$ 12	126 $\pm$ 11	114 $\pm$ 3.6
1,500 AIU + "	77 $\pm$ 14	92 $\pm$ 9.6	94 $\pm$ 9.5	113 $\pm$ 9.7	112 $\pm$ 8.8	111 $\pm$ 4.2
3,000 AIU + "	65 $\pm$ 5.6	101 $\pm$ 3.3	82 $\pm$ 11	112 $\pm$ 4.5	109 $\pm$ 6.4	104 $\pm$ 2.5

TABLE 4 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of starch $\pm$ active substance from Example 8, compound of the invention with $n = 2$

Time (mins.) after administration:	5	10	15	20	30	45
Control without starch	61 $\pm$ 3.7	68 $\pm$ 3.9	83 $\pm$ 5.3	75 $\pm$ 8.7	91 $\pm$ 5.2	84 $\pm$ 1.5
Control with starch	85 $\pm$ 7.9	120 $\pm$ 7.7	132 $\pm$ 11	140 $\pm$ 12	135 $\pm$ 5.0	128 $\pm$ 15
150 AIU + starch	82 $\pm$ 7.5	105 $\pm$ 4.2	120 $\pm$ 6.0	124 $\pm$ 7.0	134 $\pm$ 6.1	125 $\pm$ 5.2
300 AIU + "	79 $\pm$ 8.2	97 $\pm$ 2.7	105 $\pm$ 10	118 $\pm$ 6.7	119 $\pm$ 9.9	130 $\pm$ 5.9
600 AIU + "	70 $\pm$ 4.6	80 $\pm$ 8.8	94 $\pm$ 6.9	91 $\pm$ 16	103 $\pm$ 9.2	102 $\pm$ 4.2
1,200 AIU + "	70 $\pm$ 7.0	84 $\pm$ 7.7	88 $\pm$ 7.4	97 $\pm$ 5.2	97 $\pm$ 5.0	100 $\pm$ 4.3
----- P<0.05 ----- P<0.01 ----- P<0.001 against control with starch						

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TABLE 5 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of starch $\pm$ active substance from Example 15.

Time (mins.) after administration:	5	10	15	20	30	45
Control without starch	74 $\pm$ 6.2	74 $\pm$ 3.0	82 $\pm$ 9.4	71 $\pm$ 11	76 $\pm$ 5.4	90 $\pm$ 9.2
Control with starch	101 $\pm$ 8.8	135 $\pm$ 13	146 $\pm$ 4.8	151 $\pm$ 9.5	148 $\pm$ 18	156 $\pm$ 19
75 AIU + starch	102 $\pm$ 2.9	130 $\pm$ 6.9	128 $\pm$ 5.4	143 $\pm$ 11	125 $\pm$ 11	132 $\pm$ 18
150 AIU + "	98 $\pm$ 8.7	116 $\pm$ 9.0	128 $\pm$ 5.8	127 $\pm$ 4.8	115 $\pm$ 5.2	137 $\pm$ 5.2
300 AIU + "	87 $\pm$ 13	106 $\pm$ 1.8	110 $\pm$ 8.9	111 $\pm$ 2.1	108 $\pm$ 5.3	121 $\pm$ 7.2

TABLE 6 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of sucrose $\pm$ active substance from Example 16, compound with n = 5-7

Time (mins.) after administration:	15	30	45	60
Control without sucrose	58 $\pm$ 2.9	49 $\pm$ 3.3	54 $\pm$ 4.8	63 $\pm$ 5.0
Control with sucrose	107 $\pm$ 11	121 $\pm$ 6.0	132 $\pm$ 16	132 $\pm$ 6.7
25 SIU + sucrose	102 $\pm$ 11	108 $\pm$ 9.6	109 $\pm$ 7.5	101 $\pm$ 7.9
50 SIU + sucrose	92 $\pm$ 7.0	100 $\pm$ 8.3	109 $\pm$ 3.7	102 $\pm$ 6.3
100 SIU + "	95 $\pm$ 8.1	84 $\pm$ 8.7	93 $\pm$ 9.0	91 $\pm$ 4.5

----- P<0.05 ----- P<0.01 ----- P<0.001 against control with starch or sucrose

TABLE 7 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of sucrose $\pm$ active substance from Example 8, compound of the invention with n = 4-6

Time (mins.) after administration	15	30	45	60
Control without sucrose	58 $\pm$ 2.9	49 $\pm$ 3.3	54 $\pm$ 4.8	63 $\pm$ 5.0
Control with sucrose	107 $\pm$ 11	121 $\pm$ 6.0	132 $\pm$ 16	132 $\pm$ 6.7
25 SIU + sucrose	109 $\pm$ 6.4	105 $\pm$ 10	112 $\pm$ 9.1	108 $\pm$ 8.1
50 SIU + ..	103 $\pm$ 4.1	107 $\pm$ 15	102 $\pm$ 7.7	101 $\pm$ 9.2
100 SIU + ..	85 $\pm$ 8.1	94 $\pm$ 9.9	95 $\pm$ 8.5	85 $\pm$ 6.4

TABLE 8 accompanying Example 17

Blood glucose in mg % (average value $\pm$ 1s) of fasting rats at various times after oral administration of sucrose $\pm$ active substance from Example 8, compound of the invention with n = 2.

Time (mins.) after administration:	15	30	60
Control without sucrose	15 $\pm$ 4.5	92 $\pm$ 8.5	95 $\pm$ 6.4
Control with sucrose	113 $\pm$ 9.9	126 $\pm$ 20	127 $\pm$ 14
25 SIU + ..	71 $\pm$ 3.7	100 $\pm$ 7.3	107 $\pm$ 2.4
100 SIU + ..	58 $\pm$ 5.9	92 $\pm$ 4.0	98 $\pm$ 5.0

----- P<0.05 ----- P<0.01 ----- P<0.001 against control with sucrose

TABLE 9 accompanying Example 17

Blood glucose in mg% and serum insulin in μ U/ml of 2 fasting human subjects after oral administration of sucrose $\pm$ active substance from Example 8, crude inhibitor (26,000 SIU/g)

Mins. after administration:	<u>0</u>	<u>15</u>	<u>30</u>	<u>45</u>	<u>60</u>	<u>90</u>	<u>120</u>	<u>180</u>
<u>Subject A in control experiment with sucrose</u>								
Blood glucose	120	178	190	176	140	94	100	104
Serum insulin	14	33	45	37	27	17	9	13
<u>Subject A in experiment with sucrose + 1,000 SIU</u>								
Blood glucose	122	146	148	132	116	120	112	110
Serum insulin	10	20	18	18	13	14	12	10
<u>Subject B in control experiment with sucrose</u>								
Blood glucose	108	122	160	158	140	100	96	104
Serum insulin	7	14	31	21	19	10	7	13
<u>Subject B in experiment with sucrose + 1,000 SIU</u>								
Blood glucose	102	122	134	128	120	112	110	106
Serum insulin	6	13	15	13	7	8	8	10

TABLE 10 accompanying Example 17

Blood glucose in mg% (average value $\pm$ 1s) of fasting rats at various times after oral administration of sucrose $\pm$ active substance from Example 15.

Minutes after administration:	15	30	45
Control without sucrose	69 $\pm$ 5.2	84 $\pm$ 3.9	91 $\pm$ 5.9
Control with sucrose	117 $\pm$ 11	135 $\pm$ 8.1	152 $\pm$ 18
50 SIU + "	105 $\pm$ 11	123 $\pm$ 5.2	128 $\pm$ 11
100 SIU + "	89 $\pm$ 6.5	111 $\pm$ 4.1	116 $\pm$ 3.9
200 SIU + "	72 $\pm$ 4.6	95 $\pm$ 9.1	104 $\pm$ 9.6

TABLE 11 accompanying Example 17

Blood glucose in mg% (average value $\pm$ 1s) of fasting rats at various times after oral administration of maltose $\pm$ active substance from Example 8, crude inhibitor (26,000 SIU/g; 6,000 MIU/g)

Minutes after administration:	5	10	20	30	60	90
Control without maltose	69 $\pm$ 1.6	71 $\pm$ 4.6	91 $\pm$ 3.0	90 $\pm$ 9.6	101 $\pm$ 13	72 $\pm$ 5.3
Control with maltose	101 $\pm$ 5.8	138 $\pm$ 11	179 $\pm$ 10	174 $\pm$ 17	186 $\pm$ 17	148 $\pm$ 21
100 MIU + "	104 $\pm$ 5.1	108 $\pm$ 4.1	139 $\pm$ 7.6	135 $\pm$ 13	150 $\pm$ 13	132 $\pm$ 5.3
200 MIU + "	92 $\pm$ 6.6	101 $\pm$ 9.6	128 $\pm$ 8.5	128 $\pm$ 11	136 $\pm$ 6.6	126 $\pm$ 3.6

----- P<0.05 ——— P<0.01

----- P<0.001 against control with sucrose or maltose

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TABLE 12 accompanying Example 17

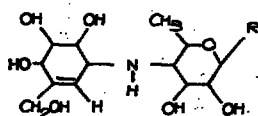
Blood glucose in mg% (average value $\pm$ 1s) of fasting rats at various times after oral administration of glucose $\pm$ active substance from Example 8, compound of the invention with $n = 2$

Minutes after administration:	15	30	45
Control without glucose	72 $\pm$ 4.6	78 $\pm$ 1.3	87 $\pm$ 6.8
Control with glucose	142 $\pm$ 12	142 $\pm$ 12	158 $\pm$ 19
30 mg + ..	135 $\pm$ 13	128 $\pm$ 5.5	132 $\pm$ 7.3
60 mg + ..	125 $\pm$ 13	118 $\pm$ 2.9	130 $\pm$ 9.0

----- P<0.05 ——— P<0.01 against control with glucose

WHAT WE CLAIM IS:—

1. Compounds which are amino-sugar derivatives of the following general formula:—



(I)

in which R is an oligosaccharide chain with one, two, three or four monosaccharide units, the said compounds being substantially free from homologous amino-sugar derivatives containing more than four monosaccharide units per molecule.

2. Compounds according to claim 1 in which R is an oligosaccharide chain with one, two or three monosaccharide units, the said compounds being substantially free from homologous amino-sugar derivatives containing more than three monosaccharide units per molecule.

3. Compounds according to claim 1 or claim 2 in which the monosaccharide units are hexose residues.

4. Compounds according to claim 2 in which the said residues are glucose residues.

5. A compound according to any one of claims 1 to 4 wherein R consists of a single monosaccharide unit.

6. A compound according to any one of claims 1 to 4 wherein R consists of two monosaccharide units.

7. A process for the production of an amino-sugar derivative in which either:
(a) a micro-organism of the strain *Actinoplanes* SE 50/110 (CBS 674.73) is cultured in an aqueous medium under aerobic conditions and a compound of the formula I or a homologue thereof containing up to seven monosaccharide units in the side chain is separated from the resultant culture; or

(b) a compound according to any one of claims 1 to 6 is produced from a homologous amino-sugar derivative which contains up to seven side chain monosaccharide units by splitting off the necessary number of monosaccharide units from the molecule microbially or by chemical or enzymatic hydrolysis, and freeing the resulting material from homologous amino-sugar derivatives containing more than four monosaccharide units per molecule.

8. A process according to claim 7(a) in which the medium contains, as a carbon source, starch, maltose, glucose, malt extract, or a mixture of two or more thereof.

9. A process according to claim 7(a) in which the medium contains as carbon source either more than 2 wt.% of starch or glucose and 0.5—2 wt.% starch.

10. A process according to claim 7 in which the micro-organism is cultured at about 28°C.



TABLE 12 accompanying Example 17

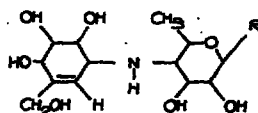
Blood glucose in mg% (average value $\pm$ 1s) of fasting rats at various times after oral administration of glucose $\pm$ active substance from Example 8, compound of the invention with $n = 2$

Minutes after administration:	15	30	45
Control without glucose	72 $\pm$ 4.6	78 $\pm$ 1.3	87 $\pm$ 6.8
Control with glucose	142 $\pm$ 12	142 $\pm$ 12	158 $\pm$ 19
30 mg + ..	135 $\pm$ 13	128 $\pm$ 5.5	132 $\pm$ 7.3
60 mg + ..	125 $\pm$ 13	118 $\pm$ 2.9	130 $\pm$ 9.0

----- P<0.05 ————— P<0.01 against control with glucose

WHAT WE CLAIM IS:—

1. Compounds which are amino-sugar derivatives of the following general formula:—



(I)

in which R is an oligosaccharide chain with one, two, three or four monosaccharide units, the said compounds being substantially free from homologous amino-sugar derivatives containing more than four monosaccharide units per molecule.

2. Compounds according to claim 1 in which R is an oligosaccharide chain with one, two or three monosaccharide units, the said compounds being substantially free from homologous amino-sugar derivatives containing more than three monosaccharide units per molecule.

3. Compounds according to claim 1 or claim 2 in which the monosaccharide units are hexose residues.

4. Compounds according to claim 2 in which the said residues are glucose residues.

5. A compound according to any one of claims 1 to 4 wherein R consists of a single monosaccharide unit.

6. A compound according to any one of claims 1 to 4 wherein R consists of two monosaccharide units.

7. A process for the production of an amino-sugar derivative in which either:
(a) a micro-organism of the strain *Actinoplanes* SE 50/110 (CBS 674.73) is cultured in an aqueous medium under aerobic conditions and a compound of the formula I or a homologue thereof containing up to seven monosaccharide units in the side chain is separated from the resultant culture; or

(b) a compound according to any one of claims 1 to 6 is produced from a homologous amino-sugar derivative which contains up to seven side chain monosaccharide units by splitting off the necessary number of monosaccharide units from the molecule microbially or by chemical or enzymatic hydrolysis, and freeing the resulting material from homologous amino-sugar derivatives containing more than four monosaccharide units per molecule.

8. A process according to claim 7(a) in which the medium contains, as a carbon source, starch, maltose, glucose, malt extract, or a mixture of two or more thereof.

9. A process according to claim 7(a) in which the medium contains as carbon source either more than 2 wt.% of starch or glucose and 0.5—2 wt.% starch.

10. A process according to claim 7 in which the micro-organism is cultured at about 28°C.



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11. A process according to claim 7 or 8 in which the desired compound is precipitated from the culture medium after decolourization, and concentrated.
12. A process according to claim 7(a) in which the medium is starch-free.
13. A process according to claim 10 in which the medium contains maltose.
14. A process according to claim 7(a) in which the medium is glucose-free and contains maltose or malt extract as a carbon source.
15. A process according to any one of claims 12 to 14 in which the micro-organism is cultured at about 24°C.
16. A process according to any one of claims 12 to 15 in which the desired compound or compounds are separated from the culture medium by adsorption on active charcoal at substantially neutral pH and desorbed therefrom by aqueous alcohol or acetone and pH 1.5—4.
17. A process according to claim 7(b) in which at least one monosaccharide unit is split off by aqueous hydrolysis at 50—100°C in 1.5 N. mineral acid over a period of 10—180 minutes.
18. A process according to claim 7(b) in which at least one saccharide unit is split off by incubation with a β -amylase or non-inhibited α -amylase, or an amyloglucosidase of microbial origin.
19. A process according to claim 7(b) in which at least one monosaccharide unit is split off by culturing a suitable micro-organism in a nutrient medium containing 1—10 wt.% of the said homologous derivative as the sole carbon source.
20. A process according to any one of claims 7 to 19 comprising separating the desired compound or compounds from undesired inhibiting materials by adsorbing the said compound or compounds from solution at pH 1—8 and an ionic strength corresponding to a conductivity of less than 10 mS, onto an acid cation exchanger in the H^+ form, and desorbing the desired compound or compounds therefrom with aqueous ammonia.
21. A process according to any of claims 7 to 19 comprising also separating the desired compound or compounds from undesired inhibiting material by adsorbing the said compound or compounds from solution at pH 1—8 and an ionic strength corresponding to a conductivity of less than 10 mS onto an acid cation exchanger in the H^+ form and desorbing the desired compound or compounds therefrom with aqueous acids.
22. A process according to any of claims 7 to 19 comprising also separating the desired compound or compounds from inert saccharides by chromatography on a cellulose-based ion exchanger.
23. A process according to claim 22 in which the ion exchanger is a phospho-cellulose from which the inhibitor is eluted by a buffer of ionic strength 2—100 mM at a pH of 2.5—8.
24. A process according to any one of claims 7 to 23 in which the individual desired compound or compounds are isolated by chromatographic fractionation on a molecular sieve.
25. A process for the production of an amino sugar derivative substantially as hereinbefore described in any of Examples 5 or 7 to 16.
26. A process for the production of an amino sugar derivative substantially as hereinbefore described in Example 17.
27. An amino sugar derivative whenever produced by a process according to any of claims 7 to 25.
28. A pharmaceutical composition for inhibiting saccharase activity and starch digestion containing as an active ingredient a compound according to any one of claims 1 to 6 and 27 in admixture with a solid or liquefied gaseous diluent or in admixture with a liquid diluent other than a solvent of molecular weight less than 200 except in the presence of a surface-active agent.
29. A pharmaceutical composition containing as an active ingredient a compound according to any one of claims 1 to 6 and 27 in the form of a sterile or isotonic aqueous solution.
30. A composition according to claim 8 or 9 containing from 0.5 to 95% of the said active ingredient by weight.
31. A medicament in dosage unit form comprising a compound according to any of claims 1 to 6 and 27 either alone or in admixture with a diluent.
32. A medicament in the form of tablets, pills, dragees, capsules, ampoules, or suppositories comprising a compound according to any of claims 1 to 6 and 27 either alone or in admixture with a diluent.
33. A medicated foodstuff or beverage comprising a nutritious carbohydrate-

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containing material and a compound according to any of claims 1 to 6 and 27.

5 34. A method of combating adiposity, hyperlipaemia, diabetes, prediabetes, gastritis, gastric ulcers, duodenal ulcers and caries, non-human animals which comprises administering to the animals an active compound according to any of claims 1 to 6 and 27 either alone or in admixture with a diluent or in the form of a medicament according to claim 1 to 2 or in the form of a foodstuff or beverage according to claim 33.

35. A method according to claim 34 in which the said compound is administered orally.

10 36. A method according to claim 35 in which the said compound is administered in an amount of 30—300,000 AIU/kg. or 1—10,000 SIU/kg. body weight per day.

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