[31] G. Becker, Z. Anorg. Allg. Chem. *430*, 66 (1977).
[32] G. Becker, M. Rössler, W. Uhl, Z. Anorg. Allg. Chem. *473*, 7 (1981).
[33] K. Issleib, H. Schmidt, H. Meyer, J. Organomet. Chem. *160*, 47 (1978).
[34] G. Becker, O. Mundt, Z. Anorg. Allg. Chem. *443*, 53 (1978).
[35] R. Appel, V. Barth, Angew. Chem. *91*, 497 (1979); Angew. Chem. Int. Ed. Engl. *18*, 469 (1979).
[36] R. Appel, V. Barth, M. Halstenberg, G. Huttner, J. Seyerl, Angew. Chem. *91*, 935 (1979); Angew. Chem. Int. Ed. Engl. *18*, 872 (1979).
[37] R. Appel, V. Barth, F. Knoll, I. Ruppert, Angew. Chem. *91*, 936 (1979), Angew. Chem. Int. Ed. Engl. *18*, 873 (1979).
[38] R. Appel, B. Laubach, Tetrahedron Lett. *1980*, 2497.
[39] R. Appel, F. Knoll, lecture delivered in Durham, see [22].
[40] R. Appel, A. Kirfel, M. Halstenberg, unpublished results.
[41] G. Becker, Chemisches Kolloquium, Universität Köln, June 24, 1981.
[42] R. Appel, V. Barth, Tetrahedron Lett. *1980*, 1923.
[43] R. Appel, U. Kündgen, Angew. Chem., in press.
[44] R. J. Thoma, R. H. Neilson, lecture delivered in Durham, see [22].
[45] E. Niecke, D. A. Wildbredt, Angew. Chem. *90*, 209 (1978); Angew. Chem. Int. Ed. Engl. *17*, 199 (1978).
[45] a) E. Niecke, D. A. Wildbredt, Angew. Chem. *90*, 209 (1978); Angew. Chem. Int. Ed. Engl. *17*, 199 (1978); b) R. Appel, J. Peters, A. Westerhaus, *ibid.*, in press; c) E. Niecke, D. A. Wildbredt, J. Chem. Soc. Chem. Commun. *1981*, 72.
[46] J. F. Nixon, lecture delivered in Durham, see [22].
[47] G. Becker, M. Rössler, E. Schneider, Z. Anorg. Allg. Chem. *439*, 121 (1978).
[48] G. Becker, H. P. Beck, Z. Anorg. Allg. Chem. *430*, 77 (1977).
[49] W. Paulen, Diplomarbeit, Universität Bonn 1980.
[50] E. Breitmaier, W. Voelter: ^{13}C-NMR Spectroscopy, 2nd ed., Verlag Chemie, Weinheim 1978, p. 72.
[51] C. Thomson, J. Chem. Soc. Chem. Commun. *1977*, 322.
[52] R. Appel, M. Halstenberg, unpublished results.
[53] R. Appel, R. Sievers, unpublished results.
[54] R. Appel, M. Halstenberg, P. Stegemann, unpublished results.
[55] R. Appel, opening lecture in Durham, see [22].
[56] I. F. Lutsenko, M. V. Proskurnina, Usp. Khim. *47*, 1648 (1978).
[57] M. J. Hopkinson, H. W. Kroto, J. F. Nixon, N. P. C. Simmons, Chem. Phys. Lett. *42*, 460 (1976).
[58] T. A. Cooper, H. W. Kroto, J. F. Nixon, O. Ohashi, J. Chem. Soc. Chem. Commun. *1980*, 333.
[59] G. Becker, G. Gresser, W. Uhl, Z. Naturforsch. *B 36*, 16 (1981).
[60] I. S. Matveev, Khim. Tekhnol. (Kiev) *1974*, 49; Chem. Abstr. *83*, 97470c (1975).
[61] S. P. Anderson, H. Goldwhite, D. Ko, A. Letsou, J. Chem. Soc. Chem. Commun. *1975*, 744.
[62] A. G. Brook, Chem. Eng. News *59*, Nr. 13, S. 18 (1981).
[63] G. Maier, G. Mihm, H.-P. Reisenauer, Angew. Chem. *93*, 615 (1981); Angew. Chem. Int. Ed. Engl. *20*, 597 (1981) and literature cited therein; P. Rosmus, H. Bock, B. Solouki, G. Maier, G. Mihm, *ibid. 93*, 616 (1981) and *20*, 598 (1981), literature cited therein.
[64] M. Yoshifuji, I. Shima, N. Inamoto, lecture delivered in Durham, see [22].
[65] Y. Kobayashi, S. Fujino, I. Kumadaki, J. Am. Chem. Soc. *103*, 2465 (1981).
[66] R. Appel, M. Halstenberg, S. Korte, unpublished results.

Chemistry and Biochemistry of Microbial α-Glucosidase Inhibitors

By Ernst Truscheit, Werner Frommer, Bodo Junge, Lutz Müller, Delf D. Schmidt, and Winfried Wingender[*]

Dedicated to Professor Herbert Grünewald on the occasion of his 60th birthday

α-Glucosidases are among the most important carbohydrate-splitting enzymes. They catalyze the hydrolysis of α-glucosidic linkages. Their substrates are—depending on their specificity—oligo- and polysaccharides. Microbial inhibitors of α-amylases and other mammalian intestinal carbohydrate-splitting enzymes studied during the last few years have aroused medical interest in the treatment of metabolic diseases such as diabetes. Moreover, they extend the spectrum of microbial secondary metabolites which comprises an enormous variety of structures. They also contribute considerably to a better understanding of the mechanism of action of α-glucosidases. These inhibitors belong to different classes of substances. Those studied most thoroughly are microbial α-glucosidase inhibitors which are members of a homologous series of pseudooligosaccharides of the general formula *(4)*. They all have a core in common which is essential for their inhibitory action, a pseudodisaccharide residue consisting of an unsaturated cyclitol unit, and a 4-amino-4,6-dideoxyglucose unit. The—in many respects—most interesting representative of this homologous series is acarbose *(5)*, a pseudotetrasaccharide exhibiting a very pronounced inhibitory effect on intestinal α-glucosidases such as sucrase, maltase and glucoamylase. The present paper will review this new field of microbial α-glucosidase inhibitors which has been studied with particular intensity during the past ten years.

1. Introduction

Natural enzyme inhibitors, especially inhibitors of hydrolases are found in many plants and animals as well as in bacteria and fungi. Some of them have been known for a long time. The ubiquitous protease inhibitors[1-4] have been studied most thoroughly with respect to their structure, function and mechanism of action. Publications on protease inhibitors of the serum[5,6] already appeared at the end of last century. Reports on natural α-glucosidase inhibitors date back to the early thirties of this century. In 1933 *Chrzaszcz* and *Janicki*[7] described a protein-like substance

[*] Dr. E. Truscheit, Prof. Dr. W. Frommer, Dr. B. Junge, Dr. L. Müller, Dr. D. D. Schmidt, Dr. W. Wingender
Chemisch-wissenschaftliches Labor Pharma der Bayer AG
Postfach 101709, 5600 Wuppertal 1 (Germany)

  0570-0833/81/0909-0744 $ 02.50/0

practically insoluble in water which is found in the malts of various kinds of grain, especially in buckwheat malts. Because of its malt α-amylase inactivating properties this substance was called "sistoamylase". Later *Kneen* and *Sandstedt*[8,9] described water-soluble preparations with protein character and α-amylase inhibiting properties which they had isolated from rye and wheat germ flour. These substances were potent inhibitors of α-amylases from human saliva and bacteria. Pancreatic amylase, *e.g.* porcine, was inhibited to a lesser degree. Potent inhibitors of α-amylase were later discovered also in other plants, *e.g.* bean seeds[10-14] and the tuberous root of Colocasia[15], and found to be proteins and glycoproteins. The α-amylase inhibitors of plant origin, especially those from wheat, have been studied very closely by several investigators[16] since about 1970. By means of special methods of isolation using aqueous-alcoholic solutions at acidic pH, preparations were obtained from wheat germ flour or preferably glutens, which proved to be potent inhibitors of both salivary and pancreatic α-amylases[17,18]. Such a preparation was even able to inhibit pancreatic amylase *in vivo*. As was demonstrated by starch loading tests carried out with rats, dogs, and healthy volunteers, the inhibitor, administered orally, reduces the postprandial hyperglycaemia[*] and hyperinsulinaemia dose-dependently[17,19]. These findings provided experimental evidence for a new concept developed by *Puls*[19,20] for the treatment of metabolic disorders such as diabetes mellitus, adiposity and hyperlipoproteinaemia, Type IV, and gave rise to a screening for α-glucosidase inhibitors of microbial origin in the course of which new interesting active substances were isolated from microorganisms of the order *Actinomycetales*[21]. Among these were inhibitors of α-glucosidases found to be members of a homologous series of complex oligosaccharides (pseudooligosaccharides)[22,23].

The wide distribution of microbial α-amylase inhibitors, especially in organisms of the genus *Streptomyces*, has been confirmed by various teams of investigators during the past few years (cf. Table 4). We have found that new methods for microbiological screening tests will lead to new substance classes with interesting biochemical properties so that the spectrum of microbial metabolites, showing a great structural variety and so far exhibiting predominantly antibiotic effects, is extended. Systematic studies which have been carried out by the *Umezawa* group[24] since the second half of the sixties have yielded similar results. These investigators searched and found above all microbial inhibitors of proteases, esterases, aminopeptidases and enzymes involved in the biosynthesis of epinephrine[24-26].

α-Glucosidases are hydrolases that occur ubiquitously in bacteria, fungi, plants and animals. By definition, they catalyze the hydrolysis of α-glucosidic linkages. Depending on their specificity, their substrates are oligo- and polysaccharides.

At present, there is practically no evidence that the microbial inhibitors of α-glucosidases dealt with in this paper have a biological function. However, on the one hand they are of pharmaceutical interest, on the other they contribute decisively to a better understanding of the mechanism of action of α-glucosidases. The inhibitors of this type have so far been studied mainly with regard to the effect on α-glucosidases occurring in the intestinal tract of mammals. Our interest, therefore, centers on α-amylase produced in the pancreas and secreted into the small intestine and also on oligo- and disaccharidases located in the brush border of the small intestine. These glucoamylase, maltase, isomaltase, and sucrase activities may function as reaction partners of microbial α-glucosidase inhibitors.

2. Amylases and Enzymatic Starch Hydrolysis

The natural substrate of the amylases is starch, the main storage form of carbohydrate in most of the higher plants. Detailed reviews have been published[27-29] on its structure, which was determined especially by enzymatic methods, and on its metabolism. Starch is composed of two polysaccharides, amylose and amylopectin. Partial molecular structures of these two constituents (generally 15—25% amylose and 75—85% amylopectin) are shown in Figure 1 and their most important properties are listed in Table 1.

Table 1. Properties of starch-type polysaccharides, according to *Manners* [28].

Property	Amylose	Amylopectin
General structure	essentially unbranched	branched
Average chain length [a]	≈ 10^3	20—25
Degree of polymerization [a]	≈ 10^3	10^4—10^5
Conversion into maltose [%]		
a) with α-amylase	≈ 100	≈ 90
b) with β-amylase	70—100	≈ 55
c) with β-amylase after pretreatment with a debranching enzyme [b]	100	≈ 75

[a] D-glucose residues. [b] Yeast isoamylase.

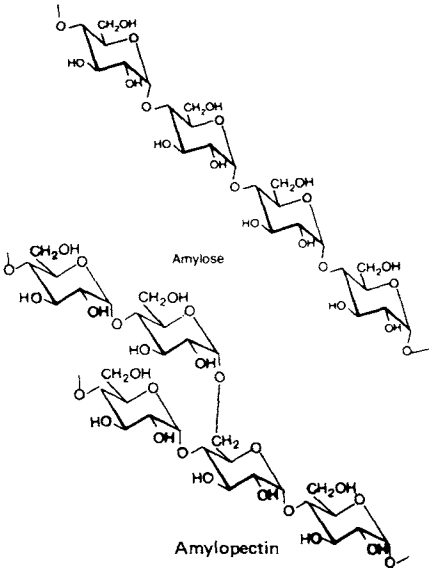


Fig. 1. Partial structures of amylose and amylopectin molecules.

[*] postprandial = occurring after a meal; hyperglycaemia = excessively increased blood glucose values; hyperinsulinaemia = excessively increased blood insulin values; adiposity = obesity.

Amylose is a linear (1→4)-α-glucan consisting of helically arranged chains of polysaccharides with an average degree of polymerization of about 1000 glucose residues. Amylopectin, on the other hand, is a branched polysaccharide the molecules of which have a much higher molecular weight (up to about 10^7). The molecules have a tree-like structure[30] consisting of two types of (1→4)-α-glucan chains with an average length of 20 and of over 50 D-glucose residues, resp. that are connected by α(1→6)-linkages. A molecule of amylopectin can contain up to 100000 D-glucose residues and 4000—5000 inter-chain α(1→6)-glucosidic linkages (and the same number of individual chains) (Figs. 1 and 2)[*].

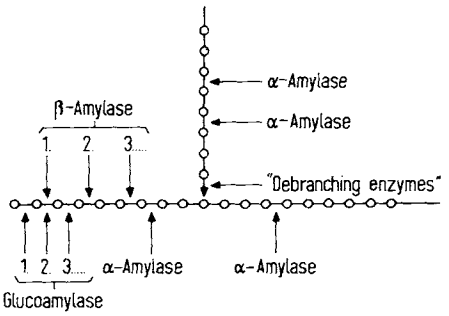
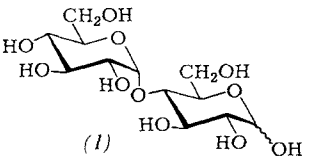


Fig. 2. Sites of action of various starch-degrading enzymes (partial structure of an amylopectin molecule).

α-Amylases (1,4-α-D-glucan glucanohydrolases, EC 3.2.1.1) attack the substrate molecule—as can be seen schematically in Figure 2 for the example of amylopectin—from inside acting as "endoenzymes", and split off maltose residues released in the α-configuration. Amylose is thus degraded mainly to maltose *(1)* by up to 100%, amylopectin by up to about 90%. In addition, slight amounts of maltotriose and glucose may result.



(1)

When amylopectin is degraded, so called α-dextrins[28] (see diagram in Fig. 3) are also formed which still contain the original inter-chain α(1→6)-glucosidic linkages not cleaved by α-amylases.

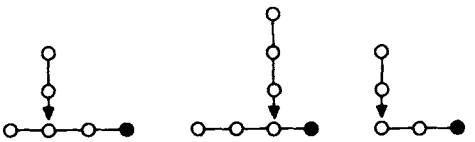


Fig. 3. Typical α-dextrin structures according to *Manners* [28]. O: α(1→4)-linked D-glucose residue; ↓: α(1→6)-inter-chain linkage; ●: free reducing group.

[*] One amylopectin molecule contains only 1 reducing glucose residue.

β-Amylases (1,4-α-D-glucan maltohydrolases, EC 3.2.1.2) are found preferentially in plants but are not produced by the mammalian organism. They act as "exoenzymes", splitting successive maltose residues (which due to inversion are released in the β-configuration) from amylose and amylopectin from the non-reducing end (cf. Fig. 2). The degradation of amylose is sometimes complete only after pretreatment with a so-called "debranching enzyme" (*e.g.* microbial isoamylase, glycogen 6-glucanohydrolase, EC 3.2.1.68) (cf. Table 1)[28] due to the presence of a very small number of branch points. The β-amylolytic degradation of amylopectin, on the other hand, remains incomplete even under these conditions (cf. Table 1), since the outermost branching sites can be neither split nor bypassed[27-29].

Glucoamylases (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3) also known as exo-α-(1→4)-glucosidases, amyloglucosidases or γ-amylases are found preferentially in microorganisms but also occur as enzyme complexes (see Section 3) in the intestinal wall of mammals. They successively split off glucose residues from the non-reducing end (Fig. 2).

The "debranching enzymes" which split α(1→6)-glucosidic linkages in the native amylopectin molecule, occur preferentially in microorganisms and plants. Corresponding enzymes of the mammalian organisms are not active in the intestinal tract. Here the intestinal oligo- and disaccharidases (Section 3) act on the α-(1→6)-glucosidic linkages of degradation products of amylopectin.

The α-amylases, the mode of action of which has already been described, are the starch-degrading enzymes investigated most thoroughly. Highly purified preparations have been obtained from a great variety of microbes, plants and animals and have been biochemically characterized. The main sources for animal α-amylases are pancreatic juice and saliva. Different forms (isoenzymes) of both human salivary amylase[31] of molecular weights between 50000 and 53000 and pancreatic amylase of hogs[32] of molecular weights between 51000 and 54000 have been

Principle of determination according to *Bernfield* [33]:

1) Hydrolysis of soluble starch, 5 min at 35 °C, pH 6.9 (0.02 M sodium glycerophosphate buffer, 0.001 M $CaCl_2$).
2) Determination of the reducing groups formed by reaction with 3,5-dinitrosalicylic acid reagent and measurement of the extinction at 540 nm.

Definition of amylase unit (AU):
1 AU = 1 μVal maltose/min.

Definition of amylase-inhibitor unit AIU:
1 AIU: amount of inhibitor which after previous incubation with the enzyme inhibits 2 AU by 50%.

Scheme 1. Activity of α-amylase.

Principle of determination according to F.I.P. [34]; modification of the test according to *Willstätter* (*R. Willstätter, G. Schudel*, Ber. Dtsch. Chem. Ges. *51*, 780 (1918)):

1) Hydrolysis of soluble starch at pH 6.8 and 25 °C (in the presence of NaCl).
2) Titration of the reducing groups with iodine in alkaline solution.

Definition of amylase unit according to F.I.P. (FIP-AU):
1 FIP-AU: that amount of enzyme which, under the test conditions, splits starch at such an initial rate that 1 micro-equivalent of glucosidic linkages is hydrolyzed per minute.

Definition of "F.I.P. amylase inhibitor unit (FIP-AIU)":
1 FIP-AIU: that amount of inhibitor which after previous incubation with the enzyme inhibits 2 FIP-AU by 50%.

Scheme 2. Activity of α-amylase.

described. Partial sequences of the polypeptide chain, made up of approximately 470 amino acid residues, are known.

Various methods are used to determine the activity of α-amylases and their inhibitors. We refer to two test methods which are briefly described in Schemes 1 and 2.

The first of these methods introduced by *Bernfeld*[33] is used frequently, though in many modified forms. The second method, the so-called "FIP-Test" is recommended by the "Commission on Enzymes" of the Fédération Internationale Pharmaceutique for the determination of activity of pancreatic α-amylase[34]. The inhibitory tests outlined in Schemes 1 and 2 have been described in detail[21]. Other tests, especially to determine the activity of α-amylase inhibitors of plant origin, have been described in detail by *Whitaker*[35] and *Marshall*[36].

3. Intestinal Oligo- and Disaccharidases, Their Substrates and the Determination of Their Activity

While after food intake pancreatic α-amylase is secreted by the pancreas into the duodenum and—apart from a partial adsorption at the intestinal wall—exerts its effect (the initiation of intestinal starch digestion) in the lumen of the small intestine, the intestinal oligo- and disaccharidases are fixed components of the cell membrane of the "brush border region" of the wall of the small intestine. These glycoside hydrolases of the intestinal wall, of which only slight amounts are released into the intestinal lumen, act mainly in a membrane-bound form. Their properties and mode of action and the molecular mechanism of action of sucrase are described in detail in a review by *Semenza*[37]. These enzymes are listed in Table 2.

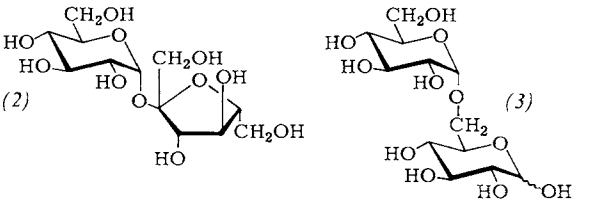
Table 2. Oligo- and disaccharidases of the small intestine [37].

Designation	EC number
1 Maltases-glucoamylases (γ-amylases)	3.2.1.20 or 3.2.1.3.
2 Sucrase-maltase	3.2.1.48 or 3.2.1.20
3 Isomaltase-maltase	3.2.1.10 or 3.2.1.20
4 α-Limit dextrinase [40]	
5 Trehalase	3.2.1.28
6 Lactase and hetero-β-glucosidase (phlorizine hydrolase)	3.2.1.23 and 3.2.1.62

Trehalase, lactase and hetero-β-glucosidase have been included for the sake of completeness.

Like the other membraneous glucosidases, maltase-glucoamylases—also known as γ-amylases—can be obtained in a homogeneous form by solubilization of an intestinal mucosa preparation with papain and subsequent chromatography. A preparation of human origin is a glycoprotein with a carbohydrate content of 32—38% and a molecular weight of the protein portion of about 220000[38]. Substrates are maltose *(1)*, maltotriose and higher α(1→4)-glucans including amylose and amylopectin, *viz.* starch. By cleaving linkages from the non-reducing end (cf. Fig. 2) glucose in the α-pyranose form is produced. The process of splitting is the faster, the smaller the substrate molecule—in contrast to the splitting of poly- and oligosaccharides by pancreatic α-amylase which is already slightly inhibited by maltose. Maltase-glucoamylases thus improve the effectivity of pancreatic amylase. These enzymes are competitively inhibited by 2-amino-2-hydroxymethyl-1,3-propanediol (tris).

The sucrase-isomaltase complex, which among the intestinal oligo- and disaccharidases has been characterized in greatest detail, can be obtained by solubilizing the mucosa material of the small intestine with either proteases (papain) or detergents (Triton X-100, sodium dodecylsulfate (SDS)). This complex is a glycoprotein with a carbohydrate content of 15% and a molecular weight for the protein component of about 220000. The complex can be split into 2 sub-units by various methods: into a sucrase-maltase and an isomaltase-maltase. Both units have an active center with sucrase and maltase specificity and with isomaltase and maltase specificity, respectively. The sucrase-isomaltase complex from the mucosa of human small intestine can be split using β-mercaptoethanol and urea[39]. The 2 sub-units are very much alike: their amino acid composition is similar. Both liberate glucose in the α-pyranose form. The sucrase-maltase complex splits sucrose *(2)*, maltose *(1)*, maltotriose and higher oligosaccharides. Substrates for the isomaltase-maltase sub-unit are, besides maltose *(1)* and isomaltose *(3)*, other oligosaccharides with α(1→6)-glucosidic linkages such as isomaltulose, isomaltotriose, α-dextrins (cf. Fig. 3) and higher limit dextrins.



Both sub-units are competitively inhibited by tris. According to recent studies by *Marshall et al.*[40], a further α-glucosidase activity of the small intestinal wall, designated limit dextrinase, which splits α(1→6)-glucosidic linkages is required in addition to the isomaltase-maltase complex.

The function of the intestinal α-glucosidases described in this section is, on the one hand, the further degradation of oligosaccharides (maltose, maltotriose, α-dextrins, isomaltose *etc.*), that are produced by pancreatic α-amylase from starch, to absorbable glucose which is introduced into the intermediary metabolism. On the other hand, they also break down oligosaccharides such as sucrose ingested with the food to absorbable monosaccharides such as glucose and fructose. *In vivo*, these processes are rather complex and have not yet been clarified in all detail. A recent review by *Semenza*[41] presents a critical evaluation of the present state of knowledge and of open questions in this field.

When determining the activities of intestinal oligo- and disaccharidases and their inhibitors, mostly enzyme preparations obtained by solubilizing mucosa of the small intestine *e.g.* from hogs[42] are used. The specificity of the test applied depends on the substrate (sucrose[23,43b], maltose[44], isomaltose[44], soluble starch[44], "dextrin" (obtained by the exhaustive degradation of glycogen with

pancreatic amylase)[45,46]). The methods of determining the activity of sucrase and its inhibition are outlined in Scheme 3[43b].

Principle of determination [43b]:

1) Hydrolysis of 0.2 M sucrose, 20 min at 37 °C, pH 6.25 (0.1 M sodium maleinate buffer).
2) Determination of released glucose, using glucose dehydrogenase reagent (in 0.5 M trisbuffer, pH 7.6), 30 min at 37 °C, and determination of absorption at 340 nm.

Principle of sucrase inhibition test [43b]:
Determination of residual activity after 10 minutes of pre-incubation of the enzyme with the inhibitor at 37 °C, pH 6.25.

Scheme 3. Activity of sucrase.

4. Survey of the Occurrence and Composition of Microbial α-Glucosidase Inhibitors

In a screening for microbial α-glucosidase inhibitors started about 10 years ago, inhibitory activities, especially against pancreatic α-amylase and small intestinal sucrase from hogs, were found in almost all genera of the order *Actinomycetales*, of which many strains were available[21,25]. These inhibitors occur especially in strains of the family *Actinoplanaceae*[*], above all of the genera *Actinoplanes*, *Ampullariella* and *Streptosporangium*. Table 3 presents some of the results of these studies[25]. The relatively high frequency of activities for *Actinoplanes* and *Streptosporangium* is striking.

Table 3. Occurrence of α-amylase and sucrase inhibitors in strains of various genera [25].

Genus	No. of strains tested	Strains active against amylase from hog pancreas	Strains active against sucrase from small intestine of hogs
Streptomyces	85	3	2
Actinoplanes	220	43	24
Streptosporangium	161	18	24

Some strains showed effects on individual α-glucosidases, the others were effective against several enzyme activities. The preparation of α-amylase inhibitors resulted in two different groups of active substances[23,25]:

a) inhibitors of a polypeptide character which are heat-labile, hard to dialyze if at all, and can be inactivated by treatment with trypsin, urea or β-mercaptoethanol.
b) inhibitors which are complex oligosaccharides and have been studied most thoroughly (see Section 5). They are stable to heat (at pH 7), acid (up to pH 2), alkali (up to pH 12) and some are dialyzable. Besides α-amylase inhibitors this group includes inhibitors of oligo- and disaccharidases of the mammalian intestinal tract.

[*] These strains, until that date hardly exposed to testing for secondary metabolite formation, were isolated by *A. Henssen* and *D. Schäfer*, Fachbereich Botanik, Universität Marburg (Germany).

The occurrence of α-amylase inhibitors, which will be described in detail in the following sections, has during the past few years been confirmed by various groups of investigators especially for *Streptomycetes sp.* (Table 4).

Table 4. Occurrence and composition of microbial inhibitors of mammalian α-glucosidases (arranged in chronological order of publication).

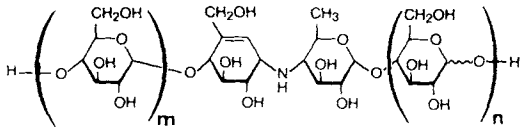
Occurrence	Composition	Specificity	Ref.
Actinomycetes of various genera	proteins, complex oligosaccharides	salivary and pancreatic α-amylases, glucoamylases, intestinal oligo- and disaccharidases	[21, 23]
Streptomyces flavochromogenes	carbohydrate-containing polypeptides (inhibitors A,B,B',C)	salivary, pancreatic amylase; bacterial α-amylases, *Rhizopus* glucoamylase	[47–50]
Streptomyces diastaticus var. amylostaticus	N-containing oligosaccharide (amylostatin, S-AI)	salivary, pancreatic amylase; microbial α-amylases, malt amylase, *Rhizopus* amylase	[51–53]
Streptomyces fradiae	acidic polypeptide, mol. weight ≈6500 (inhibitor X2)	α-amylases	[54]
Bacilli, *Streptomyces sp.*	nojirimycin, 1-deoxynojirimycin	intestinal mammalian oligo- and disaccharidases; plant and fungal β-glucosidases	[44, 55–63]
Streptomyces sp.	glucose-containing oligosaccharide, mol. weight ≈600	salivary α-amylase, glucoamylase	[64]
Streptomyces calidus	glycopeptide, rich in lysin and glucose	α-amylases, maltase and sucrase of digestive tract	[65]
Streptomyces tendae	polypeptide, mol. weight ≈7400	animal α-amylases	[66, 67]
Streptomyces sp.	N-containing oligo-saccharides ("amino sugars")	α-amylase, sucrase, maltase	[68]
Streptomyces calvus	N-containing oligosaccharides, mol. weight 950–1050, 650–700 (inhibitors TAI-A and TAI-B, resp.)	microbial and mammalian α-amylases, microbial glucoamylase	[69]
Streptomyces dimorphogenes	"amino sugar derivatives" (trestatins A, B, C)	α-amylase	[70]
Streptomyces griseosporeus	proteins, mol. weight ≈8500 (inhibitors Haim I and II)	pancreatic α-amylase	[71, 72a]
Streptomyces myxogenes	weakly basic oligosaccharides (SF-1130-X_1, -X_2, -X_3)	intestinal mammalian α-glucosidase and sucrase	[72b]

5. Acarbose and Homologous Pseudo-oligosaccharidic α-Glucosidase Inhibitors

As mentioned in Section 4 a totally new substance class of secondary metabolites marked by a very pronounced inhibitory effect mainly against intestinal α-glucosidases of mammals has been found in culture filtrates[21–23]. These inhibitors are members of a homologous series of pseudo-oligosaccharides the general formula *(4)* of which is presented in Table 5.

A characteristic of these inhibitors is that they have a core essential for their inhibitory action composed of

Table 5. Pseudo-oligosaccharidic α-glucosidase inhibitors of the general formula *(4)*.



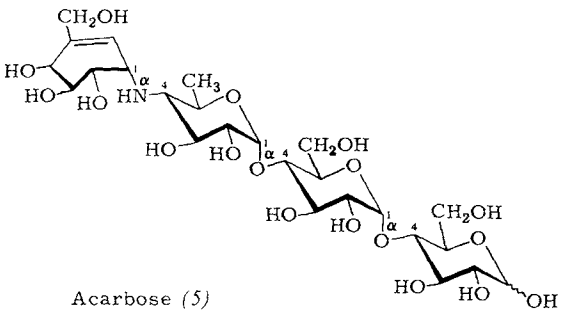
(4)

Designation	*m*	*n*	Structural formula	Ref.
"Component 2"	0	1	*(6)*	[25, 46]
"Component 3" (acarbose, BAY g 5421)	0	2	*(5)*	[23, 25, 43b, 46]
"Component 4" [a]	1	2	*(27)*	[25, 46]
"Component 5" [b]	2	2	*(28)*	[25, 46]
"Component 6" [c]	3	2	*(30)*	[25, 46]

[a] Predominant isomer with $m+n=3$. [b] Predominant isomer with $m+n=4$. [c] Predominant isomer with $m+n=5$.

a cyclitol unit (hydroxymethylconduritol residue) and a 4-amino-4,6-dideoxy-D-glucopyranose unit (4-amino-4-deoxy-D-chinovose residue). This core is linked to a varying number of glucose residues. The linkage of the individual elements is an α(1→4)-linkage as in the natural substrates (*e.g.* amylose, maltose) of the α-glucosidases. In the cyclitol unit the arrangement of the substituents is stereochemically similar to that in an α-D-glucopyranose unit.

Individual members of this homologous series have been isolated either in a homogeneous form or in the form of hardly separable isomer mixtures and been characterized in detail (Table 5). The substance investigated most thoroughly—both with regard to its microbiology[25], chemistry, biochemistry[43b, 46, 73] and pharmacology[45, 74, 100] and to its clinical use[75, 76]—is component 3 *(5)*. It was given the test designation BAY g 5421 and the generic name acarbose[45].



Acarbose *(5)*

Pseudo-oligosaccharidic α-glucosidase inhibitors were first detected in culture broths of the *Actinoplanes* strain SE 50[*]. Inhibitors of both α-amylase from porcine pancreas and of a sucrase preparation from the small intestinal mucosa of hogs were found. The inhibitory activities of the culture broths against α-amylase or sucrase can vary widely, depending on the conditions of cultivation[25].

As can be seen from Table 6, the composition of the carbon source of the culture medium is of decisive importance for the inhibitory activities produced. Media containing glucose and maltose will yield especially high titers of sucrase inhibition while media containing starch will result in a very high content of α-amylase inhibitors[25]. An amplification of this trend was achieved by the selection of suitable natural variants of the SE 50 strain. In a starch-containing medium, sub-strain SE 50/13 produces amylase inhibitor titers of around 35000 FIP-AIU/mL and only very low sucrase inhibitor activities. Strain SE 50/110, on the other hand, produces sucrase inhibitor titers of around 60 SIU/mL in a maltose-containing medium while producing an amylase inhibitor titer of only about 760 FIP-AIU/mL[25].

Table 6. Influence of carbon source in the medium on inhibitor production of the *Actinoplanes* Strain SE 50 [25].

Carbon source	Titer after 4 d		
	SIU/L	AIU/mL	$\frac{\text{SIU}}{\text{AIU}} \times 10^{-3}$
glycerol 3%	160	100	1,6
galactose 3%	1100	100	11
maltose 3%	5500	1000	5,5
cellobiose 3%	1300	220	6
glycerol 1% + glucose 2%	2700	120	22
galactose 1% + glucose 2%	2700	120	22
maltose 1% + glucose 2%	6600	200	33
cellobiose 1% + glucose 2%	2200	105	21

The diagram in Figure 4 shows how a purified inhibitor mixture is obtained which consists mainly of such members of the homologous series of the general formula *(4)* with a very pronounced inhibitory activity against α-amylase. The actinoplanes strain SE 50/13 was cultured in a starch-containing medium. The preparation obtained by fractional precipitation of the culture filtrate with methanol and ethanol inhibits α-amylases from human saliva and pancreas and from hog pancreas to a high degree[25]. This substance (designated BAY e 4609) served, *inter alia*, as a standard preparation for pharmacological[45, 77] and clinical[78] studies. The molecular weight range of this pre-

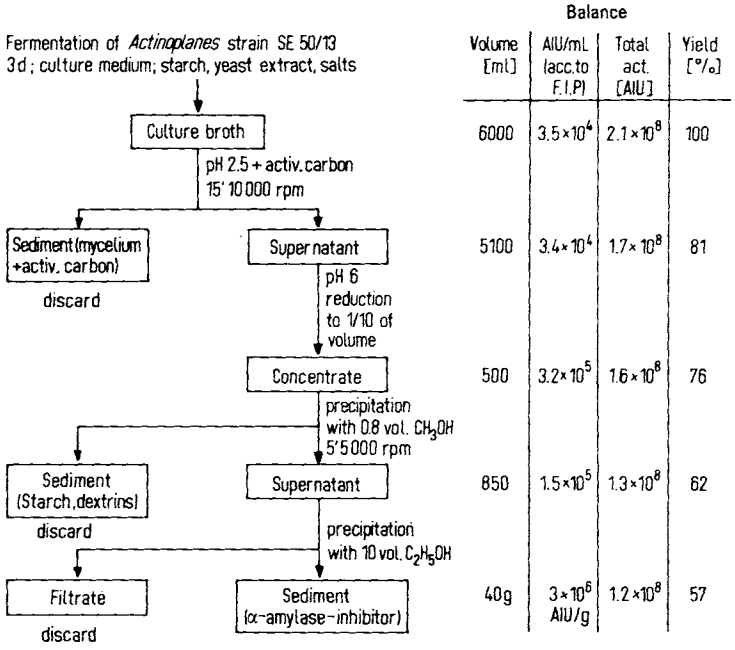


Fig. 4. Microbiological production of an α-amylase inhibitor standard preparation (BAY e 4609) [21, 85].

[*] This strain was isolated by *A. Henssen* and *D. Schäfer*, Fachbereich Botanik, Universität Marburg (Germany).

paration is determined by the conditions of precipitation (soluble in 50% CH_3OH, precipitable from a mixture of C_2H_5OH, CH_3OH and H_2O (84:8:8)).

The analysis of the oligomer distribution of this mixture consisting of oligosaccharides with inhibitory activity and inert sugars by gel chromatography (using Bio-Gel P-6)[22] resulted in a separation into two fractions with inhibitory activity with different molecular weight ranges[25,46].

The high-molecular fraction with an estimated molecular weight range of 6000 to 8000 has not been examined thoroughly yet. Rechromatography on Bio-Gel P-2 of the low-molecular weight fraction indicated a clear-cut maximum of inhibitory activity for the elution volume of a maltononaose (molecular weight 1477). This portion was shown to consist mainly of a mixture of isomers of component 7 (cf. *(4)*: $(m+n=6)$) and component 8 $(m+n=7)$. In addition, it contains small amounts of components 3 to 6 $(m+n=2, 3, 4, 5)$[22]. By re-chromatography of this fraction, using strongly acidic ion exchangers (Dowex 50 W), a preparation of the inhibitor mixture was obtained which is largely free from inert carbohydrates and exhibits a specific activity of 15×10^6 FIP-AIU/g. This corresponds to about a 5-fold enrichment of the initial material (BAYe4609)[46].

When the crude mixture of this α-amylase inhibitor (BAYe4609) is subjected to a mild acid hydrolysis with 0.5 N hydrochloric acid at 100 °C, a gradual decrease in α-amylase inhibitory activity occurs in the course of hydrolysis which is accompanied by a corresponding increase in sucrase inhibitory activity[23,43b,80]. This sucrase inhibitory activity reaches its maximum about 45 min after the start of hydrolysis after which it slowly falls to minimum values which can be interpreted as a total disintegration of the inhibitors. It was shown by thin-layer chromatography and high-pressure liquid chromatography that with consistent increase of cleavage of glucose residues the whole homologous series of inhibitory components is passed through[43b] in the course of hydrolysis. A solution with a high sucrase-inhibiting and minimal α-amylase-inhibiting activity obtained after one hour of hydrolysis has been studied closely[46].

A separation of the components with inhibitory activity from the other products of hydrolysis (mainly glucose and maltose) was achieved by adsorption on activated carbon and fractional desorption with increasing concentrations of ethanol. Four different pure components (1—4: component 1 from fraction 1, component 2 from fraction 2, *etc.*) were obtained by means of partition chromatography using cellulose (cf. Fig. 5) and after further purification by gel chromatography using Sephadex G-15. The individual components showed different inhibitory effects against sucrase, that of component 1 being very weak. Components 2—4 can—as is shown in Figure 6 by the example of component 3 (acarbose *(5)*)—be converted into the component one unit smaller by further acid hydrolysis, *i.e.* cleavage of one additional glucose residue. The end product of acid hydrolysis, component 1 *(8)*, cannot be transformed further under these conditions[23,46,79].

The complete acid hydrolysis of 100 g of the crude preparation BAYe4609 will yield about 500 mg of component 1 *(8)*. The yield of higher components produced after in-

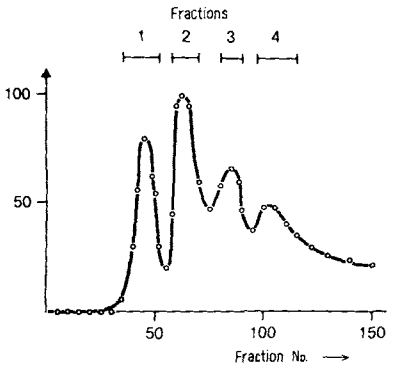


Fig. 5. Chromatography (on cellulose) of a hydrolyzate of the α-amylase inhibitor preparation BAYe4609 [46]. Solvent: pyridine/ethyl acetate/acetic acid/water (36/36/7/21); column: 1.6 × 185 cm; flow rate: 25 mL/h; fractions of 8 mL. Ordinate: Percentage of sucrase inhibition by 10 μL of each fraction.

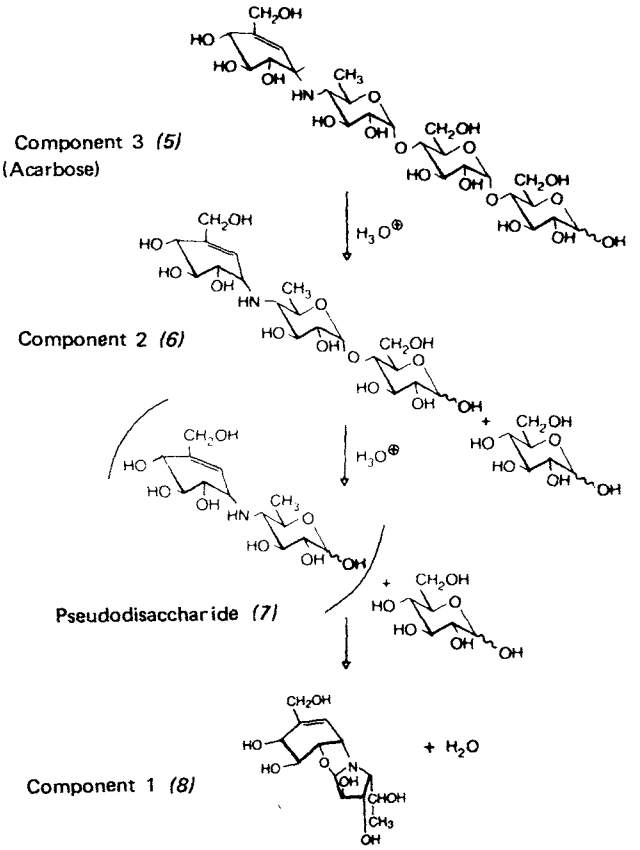


Fig. 6. Acid hydrolysis of component 3 (acarbose) *(5)* [46].

complete hydrolysis is within the same range[46]. Component 1 *(8)* is a tricyclic compound, whose structure was determined by degradation reactions, derivatization and spectroscopic analysis[46,73,79]. *(8)* is formed in the following way: after one glucose residue is split off from component 2 *(6)*, the amino sugar *(7)* released is not stable in the pyranose form. It is well known that 4-aminopyranoses such as *(7)* undergo a rearrangement to a pyrrolidine form and readily split off water in acid solution (Fig. 7). The un-

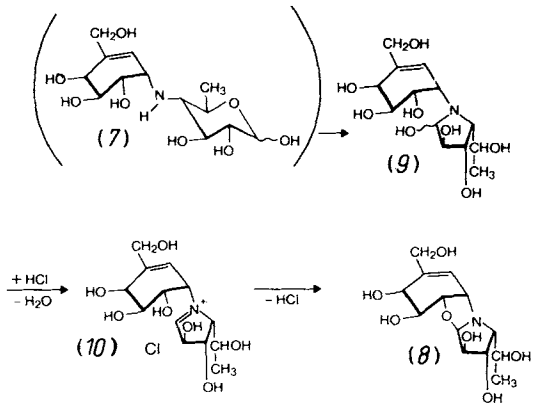


Fig. 7. Conversion of the unstable pseudodisaccharide *(7)* into a pyrrolidine derivative *(9)* and its dehydration to component 1 *(8)*.

saturated compound *(10)* is stabilized by the addition of an adjacent hydroxyl group of the cyclitol unit to the C=N double bond. The non-crystalline tricyclic product of condensation *(8)* is very easily reduced with $NaBH_4$. As a result, the 1,3-oxazolidine ring opens (Fig. 8). The resulting

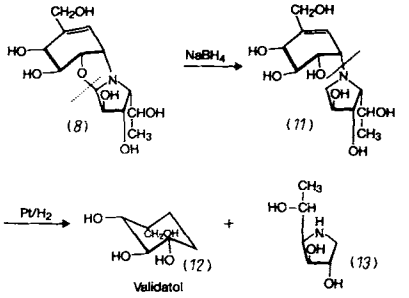
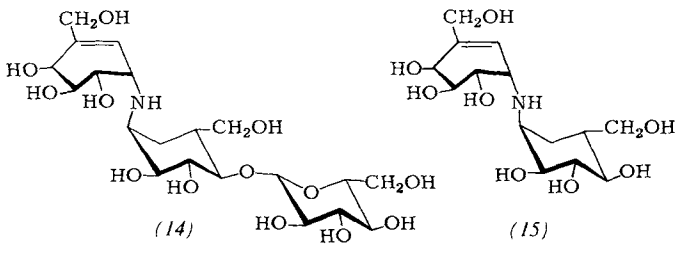
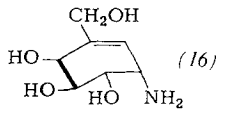


Fig. 8. Reduction of component 1 *(8)* with $NaBH_4$ to an aminocyclitol *(11)* and its catalytic hydrogenation[73].

aminocyclitol *(11)* is ineffective against α-amylase and sucrase although the cyclitol unit of the molecule has remained unchanged compared to the highly active components 2—6 of the homologous series. This means that the unsaturated hydroxymethylconduritol unit *alone* will not produce the desired inhibitory effect. The product *(11)* obtained by $NaBH_4$-reduction can be split into the cyclitol unit and the pyrrolidine unit by hydrogenolysis at a platinum contact. The main products thus obtained are validatol *(12)*[81] and the derivative *(13)*[73]. Validatol *(12)* was also obtained by degrading the antibiotic validamycin A *(14)*[81,82].



Neither validamycin A *(14)* nor validoxylamine A *(15)*[83] inhibit α-amylase or sucrase, although, as constituents of the molecule, they contain the unsaturated hydroxymethylconduritol unit. Valienamine *(16)*[84] obtained by the microbial degradation of validamycin A *(14)* with *Pseudomonas maltophila* proved to be a weak inhibitor of sucrase[73][*].



By selecting suitable strains and optimizing culture conditions the individual components of the homologous series of pseudo-oligosaccharidic α-glucosidase inhibitors can be obtained much more easily and in higher quantities[25,85,86] than by the acid hydrolysis of the preparation BAY e 4609 described above. The use of glucose or maltose in the culture medium of the *Actinoplanes* strain SE 50/110[86] will result in high sucrase inhibitor titers in the culture broths from which the inhibitors can be adsorbed by activated carbon.

Desorption with successively increasing concentrations of ethanol led to a crude preparation which was purified further by ion-exchange chromatography[46] (Fig. 9). Frac-

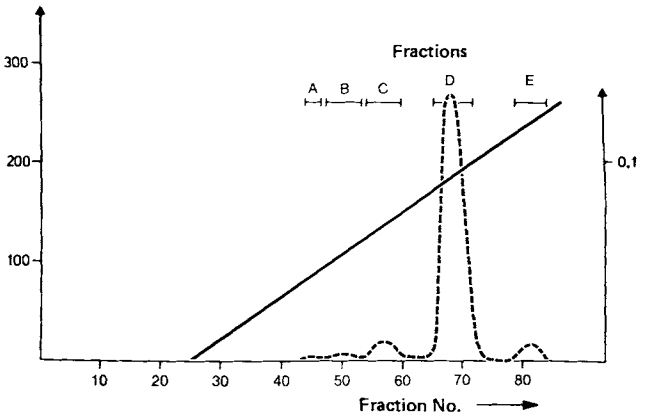


Fig. 9. Ion exchange chromatography of a crude sucrase-inhibitor preparation of the strain SE 50/110 [46]. Dowex 50 W × 4 $H^{\oplus}$ (200—400 mesh); column: 0.9 × 60 cm; elution with 10^{-3} N HCl up to fraction No. 20; then linear gradient with increasing NaCl concentrations in 10^{-3} N HCl; flow rate: 30 mL/h, fractions of 7.5 mL; Left ordinate: SIU/mL (- - -); Right ordinate: NaCl concentration (M) (——).

tions C to E are partly identical with components 2—4 (obtained by acid hydrolysis of BAY e 4609). The main fraction, approximately 80% of the sucrase inhibitors obtained from this fermentation (Fraction D, Fig. 9), proved to be identical with component 3 (acarbose) *(5)*. The yield of acarbose from fermentation was around 600 mg/L of culture broth. Fraction E was found to have one glucose unit less and identified as component 2. Fraction C has the same composition as component 4 and fractions A and B contain isomeric mixtures of the higher components 6 and 5.

Further information about the constituents essential for α-glucosidase inhibition in acarbose and in the other components was provided by hydrogenolysis with Pt/H_2, during which the cyclitol residue can be removed by cleaving the C—N bond in allyl position to the double bond without changing the remaining molecule[73]. The basic trisac-

[*] For inhibition of other α-glucosidases see [84b].

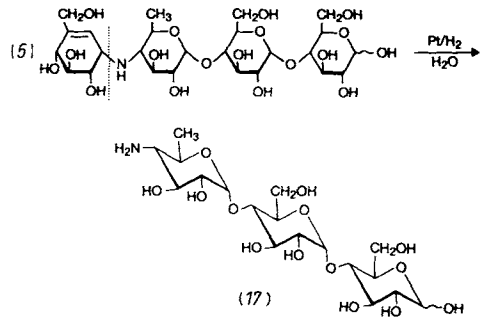


Fig. 10. Hydrogenolytic cleavage of the basic trisaccharide *(17)* during the catalytic hydrogenation of acarbose *(5)* [73].

charide obtained in this way *(17)*[*] has no inhibitory activity against α-amylase or sucrase, which means that the amino sugar *alone* is not essential for the inhibitory effect. As previously mentioned, the unsaturated cyclitol residue alone does not suffice for the inhibitory effect, either.

This leads us to the conclusion that the pseudodisaccharide unit composed of both constituents is essential for the effect. The splitting of the C—N bond in allyl position to the double bond is only one, though preferred, possibility of molecular reaction. The two C—O bonds in allyl position to the double bond are easy to split by hydrogenolysis, too (Fig. 11). Moreover, the double bond is saturated

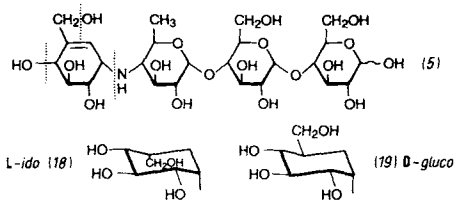


Fig. 11. Possible reactions of acarbose *(5)* during catalytic hydrogenation with maintenance of the pseudotetrasaccharide structure [73].

during hydrogenation. This will result in the formation of two diastereomeric products with an L-*ido* *(18)* or D-*gluco*-configuration *(19)*. Since there may be several of these reactions at the same time, it is easy to imagine how complex a product mixture is obtained by catalytic hydrogenation of acarbose *(5)*. The hydrogenation mixture was first separated into basic and non-basic constituents by fractionation on cation exchangers in the $H^{\oplus}$ form. Each of the two main fractions was then purified further by chromatography.

[*] *(17)* was acetylated, split into the three monosaccharide constituents by acetolysis and thus correlated to known compounds [73].

The basic reaction products (Fig. 12) isolated were, apart from the ineffective trisaccharide *(17)* mentioned, two products showing a pseudotetrasaccharide structure which had resulted from simple saturation of the double bond in the cyclitol unit. The compound *(20)* with an L-*ido*-configuration in the cyclitol unit had no inhibitory effect against either α-amylase or sucrase any more, while the other compound with a D-*gluco*-configuration in the cyclitol unit was ineffective against α-amylase but had a very pronounced effect on sucrase. A further product of hydrogenation *(22)* in which the primary hydroxyl group of the cyclitol residue had been additionally removed by reduction had no inhibitory effect on either of the enzymes.

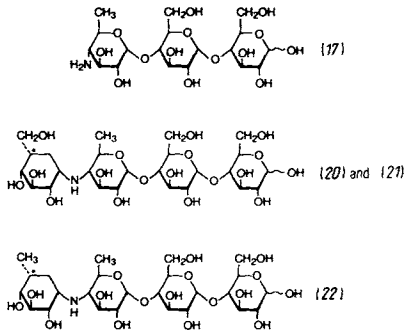
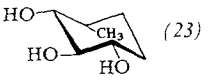


Fig. 12. Basic reaction products of the catalytic hydrogenation of acarbose *(5)* [73].

Consequently, the double bond in the cyclitol unit is—quantitatively—important for the effect. The non-basic products were examined by thin-layer chromatography and found to be derivatives of the cyclitol.

Validatol *(12)* and 7-deoxyvalidatol[81] *(23)* were isolated and identified[73].



The higher components were also subjected to hydrogenolysis in the form described. As can be seen in Figure 13,

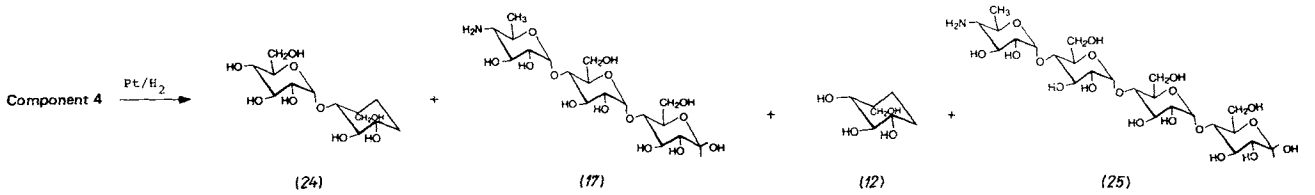
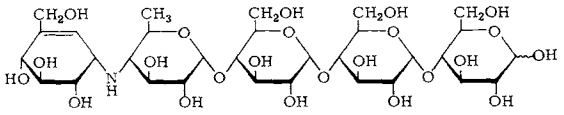
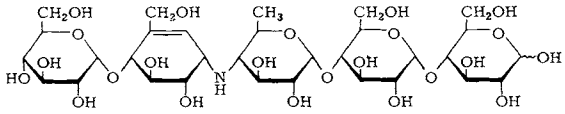


Fig. 13. Catalytic hydrogenation of component 4 [73].

"component 4" yields mainly validatol glucoside *(24)* in addition to the basic trisaccharide *(17)* mentioned. However, validatol *(12)* and a basic tetrasaccharide *(25)*[46] are also obtained. Component 4 thus consists of two isomers *(26)* and *(27)*. The isomer with the terminal cyclitol residue *(26)* constitutes only a small proportion. A separation of both isomers was achieved using strongly acidic ion exchangers.

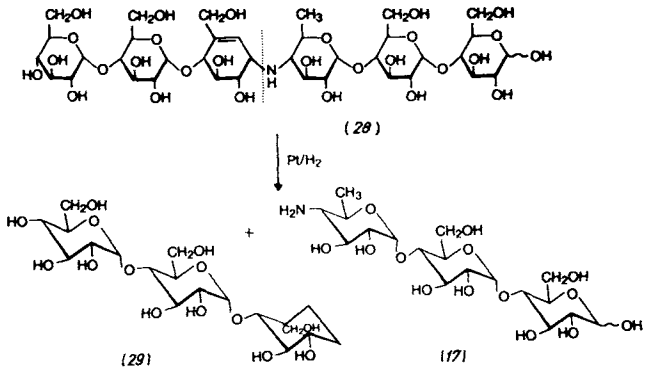


(26)



(27)

Both have inhibitory activity against α-amylase and sucrase, isomer *(26)* being slightly more effective. Components 5 and 6 (cf. Table 4) were also found to be isomer mixtures. When the main isomer *(28)* of component 5 is subjected to hydrogenolysis, validatol maltoside *(29)* and the basic trisaccharide *(17)* are formed (cf. Fig. 14).



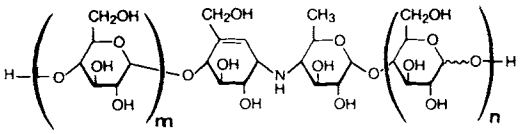
(28) (29) (17)

Fig. 14. Catalytic hydrogenation of the predominant isomer *(28)* of component 5 [73].

These data relating to the constitution of individual members of the homologous series of pseudo-oligosaccharidic α-glucosidase inhibitors of the general formula *(4)* have been confirmed and extended by reactions of enzyme degradation using β-amylase which is inhibited by the individual components to a much lesser degree than other α-glucosidases. As is shown in Figure 15, a maltose residue is split off from each of the two main isomers *(28)* and *(30)* of the isomer mixtures contained in components 5 and 6. Further products of reaction are component 3 (acarbose) *(5)* and the component 4 isomer *(27)*.

6. Inhibitory Spectrum of Acarbose and of Homologous Pseudo-oligosaccharides

The individual components of the homologous series described in detail in the previous Section differ with regard to their inhibitory activities against α-amylase from porcine pancreas and sucrase from hog small intestine (Fig. 16).



(4), m+n = 1-7

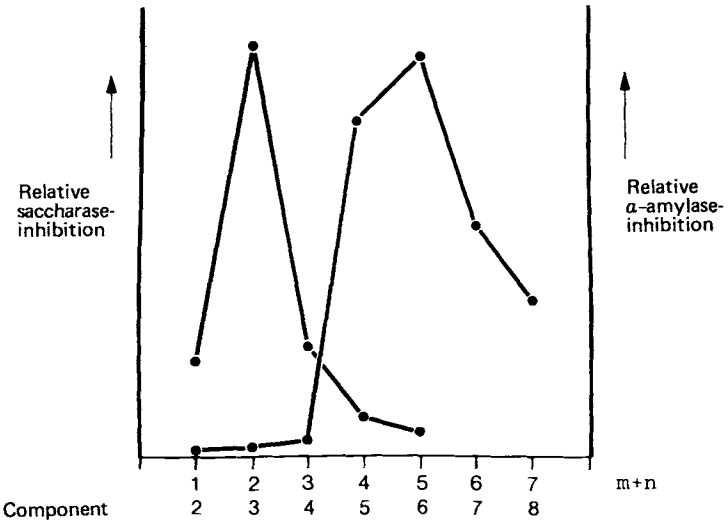
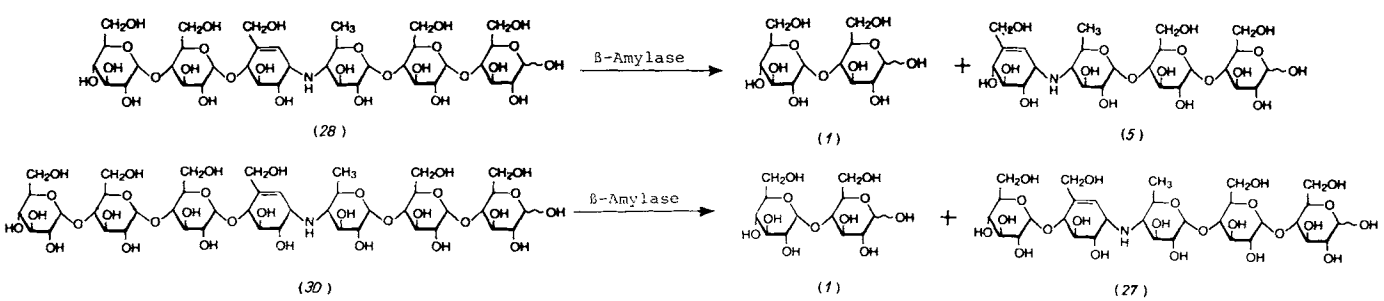


Fig. 16. Relative inhibition of sucrase and α-amylase by "components 2–8" [43].

The highest specific inhibitory activity against α-amylase is exhibited by a component 6 preparation with minimum inhibitor activity against sucrase. (The inhibition of α-amylase by the component 6 preparation is linear up to 70%. By extrapolation the amount of inhibitor for the 100% inhibition can be determined and a 1 : 1 stoichiometric relationship between enzyme and inhibitor is estimated.)

The inhibitory activity against α-amylase decreases with the higher components, while component 3 (acarbose) *(5)* shows maximum inhibitory activity against sucrase (Fig. 16). Table 7 lists the inhibitory activities of different components against α-amylase from porcine pancreas and the intestinal oligo- and disaccharidases from hog small intestine. As a measure of inhibitory activity, that amount of inhibitor is given which produces a 50% inhibition under test conditions. From Table 7 it is obvious that component 3



(28) (1) (5)

(30) (1) (27)

Fig. 15. Enzymatic hydrolysis of component 5 (predominant isomer *(28)*) and component 6 (predominant isomer *(30)*) using β-amylase (sweet potato) [46].

Table 7. Amounts (in ng) of acarbose *(5)* and homologues of the general formula *(4)* [45] necessary for inhibition (ID_{50} [a]) of the activity of intestinal α-glucosides *in vitro* [b].

Component	No. of glucose residues $m+n$ in *(4)*	Maltase 10 mU [c]	Isomaltase 10 mU	"Dextrinase" 29 mU	Glucoamylase 10 mU	α-Amylase 100 mU	Sucrase 10 mU
2	1	100	43500	205	83	855	500
3 (acarbose)	2	15	42000	64	9.3	680	74
4	3	80	54500	93	39	1135	455
5	4	236	> 100000	100	110	4.7	700
6	5	1000	⩾ 100000	93	410	2.8	700
BAY e 4609	7–30	8250	⩾ 100000	810	8000	14.5	60000

[a] Amount of inhibitor in ng per test, required for 50% inhibition. [b] Assays for glucose-releasing enzymes: Pre-incubate 0.01 ml inhibitor solution in multiple dilutions with 0.1 mL enzyme preparation for 10 minutes, incubate with 0.1 mL substrate solution, stop with 1 mL glucose dehydrogenase reagent in 0.5 M tris-buffer, develop for 30 min for determination of glucose and determine absorption at 340 nm. Substrate solutions and incubation periods: 0.4 M sucrose for 20 min, 50 mM maltose for 10 min, 40 mM isomaltose for 10 min, 5% soluble starch for 20 min. Assay for destrinase: Pre-incubate 0.01 ml inhibitor solution in multiple dilutions with 0.1 ml enzyme preparation for 10 minutes, incubate with 0.1 mL 5% limit dextrin solution for 20 minutes, mix with 0.5 mL dinitrosalicylic acid reagent [33], heat to 95 °C for 5 min, dilute with 1.5 mL water and determine absorption at 540 nm. Assay for α-amylase: Pre-incubate 0.01 mL inhibitor solution in multiple dilutions with 0.2 mL enzyme preparation (α-amylase from hog pancreas) for 10 min, incubate with 0.2 mL 5% starch solution for 5 min, mix with 0.5 mL dinitrosalicylic acid reagent [33], heat to 95 °C for 5 min, dilute with 2.5 mL water and determine absorption at 540 nm. [c] 1 U is that amount of enzyme which converts 1 μmol of substrate per min under standard conditions. 1 U ≈ 16.67 nkat.

(acarbose) *(5)* has the highest specific inhibitory activity also against the other α-glucosidases located in the wall of the small intestine. The isomaltase activity of the enzyme preparation from the small intestine of hogs is inhibited to a very small degree by the lower components (in comparison with nojirimycin and 1-deoxynojirimycin (see Section 8, Table 10)) and practically not at all by the higher components. However, the activity of the enzyme preparation referred to as "dextrinase" (substrate: glycogen degraded to limit dextrins by α-amylase from porcine pancreas) is inhibited to a high degree.

A number of bacterial, fungal and plant enzymes (cf. Table 8) were tested for inhibition by acarbose *(5)*[87]. Table 8

Table 8. Inhibitory spectrum of acarbose *(5)* for enzymes from bacteria, fungi and plants [87].

Enzyme	Origin	Substrate	Inhibition [a]
α-amylase	*B. subtilis*	starch	+
α-amylase	*A. oryzae*	starch	+
α-amylase	barley malt	starch	+
β-amylase	"sweet potato"	starch	−
β-amylase	B. polymyxa	starch	−
glucoamylase	A. niger	starch	+
invertase	*Saccharomyces sp.*	sucrose	−
α-glucosidase	*Saccharomyces sp.*	maltose	+
β-glucosidase	almonds	cellobiose	−
β-galactosidase	*Saccharomyces fragilis*	lactose	−
dextran sucrase	*Leuconostoc mesenteroides*	sucrose	−
pullulanase	*Aerobacter aerogenes*	pullulan	−
isoamylase	*Cytophaga sp.*	glycogen	−
cyclodextrin-glucosyl transferase	*B. macerans*	starch	+

[a] Inhibition: +, no inhibition: −.

indicates that α-amylases from *Bacillus subtilis* and *Aspergillus oryzea* and glucoamylase from *Aspergillus niger* and α-glucosidase from yeast *(Saccharomyces sp.)* are inhibited. As can be seen from Table 9 glucoamylase of *Aspergillus* origin is the most sensitive of all enzymes tested in this study[87]. Furthermore, cyclodextrin-glucosyl-transferase is inhibited, while muscle phosphorylase is not affected[87].

By reacting the reducing glucose unit of acarbose *(5)* a number of semisynthetic glycoside derivatives were produced and tested[73]. In a few cases an increase of up to a factor of three in the inhibitory activity against sucrase was found, but none of the derivatives offered any convincing advantages over acarbose[73]—especially when considering their costly production.

Table 9. Concentrations of acarbose *(5)* required for a 50% inhibition of various α-glucosidases [87].

Enzyme	Origin	Concentration of inhibitor [μg/mL]
α-Amylase	"Bacterial saccharifying"	0.3
	"Bacterial liquefying"	40.9
	Aspergillus oryzae	180.0
	Porcine pancreas	3.8
	Barley malt	83.5
Glucoamylase	*Aspergillus niger*	0.04
α-Glucosidase	*Saccharomyces sp.*	70.0

7. Further Pseudo-oligosaccharadic α-Amylase Inhibitors

A short time ago microbial α-amylase inhibitors became known from the patent literature which are obviously also members of a homologous series and assumed to have the same "core" as the pseudo-oligosaccharides (acarbose series) (Section 5). Three basic amino sugar derivatives were isolated from culture broths of *Streptomyces dimorphogenes* NR-320-OM7HB and NR-320-OM7HBS. These derivatives, which were separable by high-pressure liquid chromatography, were called trestatins A *(31)*, B *(32)* and C[70]. Their molecular weights as determined by osmometry are 1470, 975 and 1890, respectively. The structures proposed for trestatins A and B on the basis of spectroscopic analysis on the one hand and examinations of products of hydrolysis (4 N HCl, 3 h at 80 °C) on the other are shown in Figure 17.

The products of hydrolysis of the three trestatins are glucose and an amine ($pK_a = 3.9$) of the empirical formula $C_{13}H_{21}NO_7$[*]. All three trestatins have one trehalose residue as a common structural characteristic. Trestatin A *(31)*

[*] "Component 1" *(8)* has the same molecular formula.

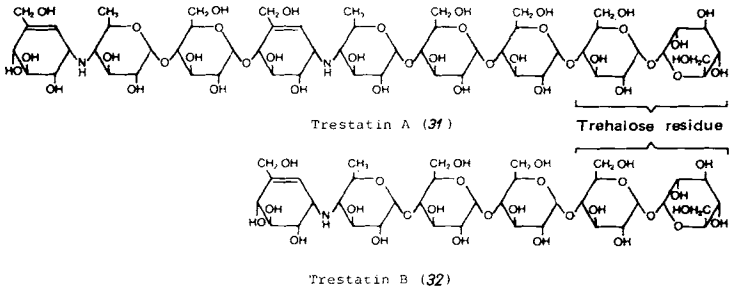
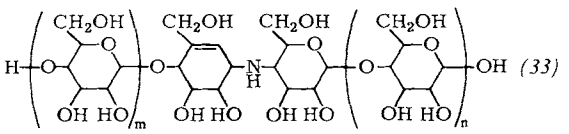


Fig. 17. Proposed structures of trestatins A *(31)* and B *(32)* [70].

contains two and trestatin C three pseudodisaccharide residues per molecule. Both show relatively high specific inhibitor activity against pancreatic α-amylase from hog (7.1×10^7 IU/g and 4.9×10^7 IU/g, respectively)[*]. Trestatin B *(32)* has only one pseudodisaccharide residue per molecule and a lower specific activity of 1.5×10^6 IU/g[*].

In a patent application[68] inhibitors of α-glucosidase are described which are also members of a homologous series of the general formula *(33)*. The microorganisms producing these inhibitors are said to be strains of the genus *Streptomyces* (*e.g.* A 2396)[68]. The only structural difference from the acarbose series of the general formula *(4)* is that the 4-amino-4,6-dideoxyglucose residue is replaced by a 4-amino-4-deoxyglucose residue. In the patent application mentioned above[68] amino sugars of the general formula *(33)* with $m = 0$ to 8, $n = 1$ to 8 are claimed, $m + n$ having a value of 1 to 8. The structure of these inhibitors was derived mainly from spectroscopic data. The higher compounds of the homologous series are more effective against α-amylase than the lower ones. Furthermore, inhibitory action on sucrase and maltase is mentioned.



Murao et al.[51–53] carried out detailed investigations on a pseudo-oligosaccharidic α-amylase inhibitor (amylostatin, S-AI) isolated from culture broths of *Streptomyces diastaticus subsp. amylostaticus* No. 2476. Its structure has not yet been completely identified, but shows a striking similarity to members of the homologous acarbose series of the general formula *(4)*. This inhibitor was isolated by chromatography followed by purification *via* an enzyme-inhibitor complex with "bacterial liquefying α-amylase" (BLA)[52]. A molecular weight of about 1500 was determined. S-AI *(34)* is called a "substrate analog inhibitor"[53]. Its inhibitory spectrum covers not only human salivary amylase and pancreatic amylase from hogs but also a number of bacterial and fungal α-amylases. Moreover, *Rhizopus niveus* glucoamylase and malt α-amylase are inhibited, while mammalian intestinal sucrase, β-amylase of plant origin and a number of microbial α-glucosidases are not. S-AI *(34)* has a reducing terminal glucose residue and five more glucose residues connected by 1,4-glucosidic linkages. Two maltose residues can be split off with β-amylase. The remaining molecule was named "glucosyl-S-AI-X-glucose"[53]. A further derivative, S-AI-XG[72] (molecular weight approximately 500) was obtained from S-AI *(34)* by enzymatic and/or chemical methods. This derivative consists of that part of the molecule containing nitrogen and a reducing glucose residue. Figure 18 shows a schematic attempt at representing the structure[53] of S-AI *(34)*, according to which the active center of BLA interacts with 8–10 glucose residues and the catalytic center of the enzyme "fits" the unknown part of the molecule S-AI-X of S-AI *(34)* which is obviously essential to the inhibitory effect.

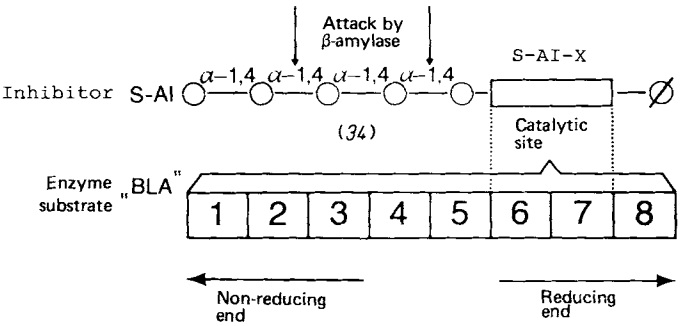


Fig. 18. Structure suggested for the microbial α-amylase inhibitor S-AI *(34)* and postulated binding mechanism between S-AI and "bacterial liquifing amylase (BLA)" according to *Murao* and *Ohyama* [53] (∅: reducing glucose residue).

Additional N-containing oligosaccharidic α-amylase inhibitors, TAI-A and TAI-B, were isolated from culture broths of *Streptomyces calvus* TM-521[69]. These inhibitors have a molecular weight of 950 to 1050 and 650 to 700, respectively, and contain two or several glucose residues and, as a basic residue, probably an amino sugar unit. TAI-A and TAI-B differ from the compounds of the homologous acarbose series of the general formula *(4)* by the absence of methyl groups. Their inhibitory spectrum covers human salivary amylase, porcine pancreatic amylase, *Rhizopus niveus* glucoamylase, Taka amylase[*] (only TAI-A) and "bacterial saccharifying α-amylase" but not BLA.

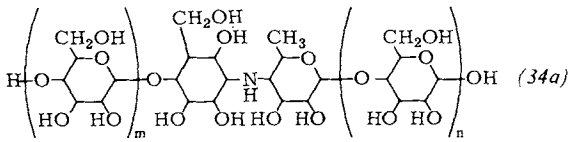
A glucoamylase and α-amylase inhibitor, NCGAI, was isolated from culture broths of *Streptomyces sp.* No. 33[64]. Its molecular weight is approximately 600 and it is assumed to consist mainly of glucose residues. NCGAI exhibits a pronounced inhibitory effect on glucoamylase while inhibiting pancreatic amylase and BLA to a lesser degree.

A short while ago a patent application became known[72a] in which three α-glucosidase inhibitors (isolated from *Streptomyces myxogenes* SF-1130, ATCC 31305) of the general formula *(34a)* are described. They are said to be weakly basic oligosaccharides. Apart from their inhibitory activity against mammalian intestinal α-glucosidase and sucrase, these substances are referred to as antibiotics

[*] Because of different test conditions these values are not comparable with those stated in Section 5.

[*] Taka amylase is a commercially available crude product from cultures of *A. oryzae* which degrades starch to glucose.

SF-1130-X_1, -X_2 and -X_3. SF-1130-X_3 (molecular weight *ca.* 830) is a particularly potent inhibitor of α-glucosidase.



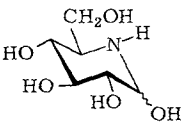
(34a)

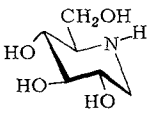
Antibioticum SF-1130-X_1, m + n = 5
Antibioticum SF-1130-X_2, m + n = 4
Antibioticum SF-1130-X_3, m = o, n = 3

8. Nojirimycin and 1-Deoxynojirimycin

Nojirimycin *(35)* was first described as an antibiotic produced by *Streptomyces roseochromogenes* R-468 and *Streptomyces lavendulae*-SF-425[56,58].

(35)





(36)

1-Deoxynojirimycin *(36)* was obtained from *(35)* by catalytic hydrogenation at the platinum contact or by reduction with $NaBH_4$[56,58]. There are also fully synthetic ways to prepare nojirimycin[58] and 1-deoxynojirimycin[57]. Later *(35)* was found to be a potent inhibitor of β-glucosidases (emulsin, fungal β-glucosidases)[59,60]. *(36)* is a much weaker inhibitor of emulsin than *(35)*[59]. *(35)* also inhibits microbial α-glucosidases, but to a much lesser degree than β-glucosidases[59,60].

A screening for inhibitors of intestinal mammalian α-glucosidases reveals that *(35)* and *(36)* are also produced by many strains of the genus *Bacillus*[44,55]. After 4- to 6-day fermentation in nutrient solutions usually containing starch or maltose, culture filtrates with relatively high titers of sucrase inhibitor units were obtained from the strains *B. amyloliquefaciens* (DSM 7), *B. polymyxa* (DSM 365), *B. subtilis* (DSM 704)[*] and *B. subtilis var. niger* (DSM 675). The inhibitors were bound to strongly acidic cation exchangers ($H^{\oplus}$ form) and subsequently eluted with aqueous ammonia. They were purified further by chromatography using CM-cellulose and gel-filtration using Sephadex LH-20. From the concentrated fractions with inhibitory activity, *(36)* crystallized in the form of colorless scales (m.p. 206 °C). *(36)* was also isolated from mulberry tree leaves[61] and called moranoline. Later *(36)* was also obtained by fermentation of the *Streptomyces lavendulae* strains SEN-158[62] and *subsp. trehalostaticus* No. 2882[63]. *(35)* and *(36)* were found to be potent inhibitors of intestinal oligo- and disaccharidases of mammals (Table 10)[44]. Pancreatic α-amylase is practically not inhibited. It was recently discovered that *(36)* is also a potent inhibitor of trehalases (rabbit and *Chaetomium aureum* MS-27) and of *Rhizopus niveus* glucoamylase and *exo*-β-1→3-glucanase *(Penicillium)*[63].

[*] This strain was isolated by *U. Heber*, Botanisches Institut, Universität Düsseldorf (Germany).

Table 10. Molar concentrations of nojirimycin *(35)* and 1-deoxynojirimycin *(36)* required for a 50% inhibition of intestinal α-glucosidases [44].

Inhibitor	Sucrase	Maltase	Isomaltase	Glucoamylase
Nojirimycin	5.6×10^{-7} M	1.7×10^{-6} M	2.5×10^{-7} M	7.6×10^{-7} M
1-Deoxynojirimycin	2.2×10^{-7} M	1.3×10^{-7} M	1.3×10^{-7} M	9.6×10^{-8} M
mU Enzyme in 0.21 mL incubation volume at 37 °C	11.1	11.8	11.5	10.1

Assays: Pre-incubate 0.01 mL inhibitor solution in multiple dilutions with 0.1 mL enzyme preparation for 10 min, incubate with 0.1 mL substrate solution, stop with 1 mL glucose dehydrogenase reagent in 0.5 M tris-buffer, develop during 30 min for determination of glucose and determine absorption at 340 nm. Substrate solutions and incubation periods: 0.4 M sucrose for 20 min, 50 mM maltose for 10 min, 40 mM isomaltose for 10 min, 5% soluble starch for 20 min. Nojirimycin is released from the bisulfite adduct using barium hydroxide, determined quantitatively using glucose dehydrogenase reagent and used immediately for the inhibition test.

9. Carbohydrate-Containing Polypeptides as Inhibitors of α-Amylase

Ueda et al. obtained carbohydrate-containing, peptide-like substances from culture broths of *Streptomyces sp.* No. 280, a variant form of *Streptomyces flavochromogenes*. These crude substances were found to inhibit quite a number of α-glucosidases, glucosyl transferases and phosphorylases (cf. Table 11)[47-50]. By combining several methods of separation (paper chromatography, paper electrophoresis) it was possible to isolate four homogeneous glycopeptide fractions A, B, B′ and C[49]. The carbohydrate portion consists mainly of glucose and the molecular weights range from 1300 (for C) to 4000. In a purified form the inhibitors had different activities against the enzymes listed in Table 11[49].

Recently the authors were able to show that the carbohydrates contained in the inhibitors are degraded enzymatically by *Streptomyces* amylase in the course of culturing the organism (in a medium containing 3% oatmeal)[50]. An additional proteolysis during fermentation cannot be excluded. An amylase preparation was isolated from the inhibitor-producing strain and used for degradation experiments with a crude inhibitor obtained after fermentation for 24 hours (probably glycopeptide fraction A, molecular

Table 11. Enzymes which are inhibited by a crude α-glucosidase inhibitor preparation of *Streptomyces flavochromogenes* [47-50].

Enzyme	Inhibition
Glucoamylase (Rhizopus)	strong
α-Amylase (saliva, human)	strong
α-Amylase (pancreas, human)	strong
α-Amylase (Bacillus)	weak
α-Amylase (Bacillus, "bacterial saccharogenic")	strong
α-Amylase (Aspergillus)	weak
α-Glucosidase (sucrase, intestinal mucosa of rat)	strong
α-D-Glucosidase (mucor)	strong
α-D-Glucosidase (yeast)	strong
Cycloamylose glucosyltransferase	strong
Phosphorylase A (rabbit muscle)	strong
Phosphorylase (potato)	strong

weight approximately 4000). The products of hydrolysis were mainly maltose besides traces of maltotriose and glucose. The inhibitory activity of the resulting "residual inhibitors" remained practically unchanged against *Rhizopus* glucoamylase while slightly decreasing against amylase from hog pancreas and sucrase from rat small intestine. The inhibitory activity against phosphorylase A from rabbit muscle was lost almost completely. This effect thus appears to be dependent to a high degree on the chain length of the carbohydrate unit. Amylase from porcine pancreas and Taka-amylase showed a similar degrading effect on the inhibitor as *Streptomyces* amylase. The above-mentioned glycopeptide inhibitors A, B, B′ and C must therefore be regarded as multiple forms of a native inhibitor formed by bioconversion.

In a patent application[65] a glycopeptide obtained from culture broths of *Streptomyces calidus* DS 26320 and having a molecular weight between 10000 and 20000 is described. This preparation inhibits pancreatic amylase and the sucrase and maltase activities of an enzyme preparation from rat small intestine. Acid hydrolysis yields lysine and monosaccharides consisting mainly of glucose.

10. Protein Inhibitors

An inhibitor of mammalian α-amylases (HOE 467), an oligopeptide having a molecular weight of approximately 7400, was isolated from the culture broths of *Streptomyces tendae*[66,67a]. The inhibition of α-amylase is irreversible. The only lysine residue in the molecule is possibly part of the active center of the inhibitor. Later, the inhibitor was separated into two fractions (A and B) and the amino acid sequence of the fraction HOE 467 A was elucidated[67b].

Two further very similar protein inhibitors free from carbohydrates and of a molecular weight of approximately 8500, Haim I and II[*], were isolated from culture broths of *Streptomyces griseosporeus* (probable identity) YM-25[71,72]; they were isolated by precipitation with ammonium sulfate and subsequent chromatography. The inhibitors show about the same specific activity against α-amylase from porcine pancreas. They differ electrophoretically and in their isoelectric points (pH 4.0 and 3.8, respectively). In addition, pancreatic amylases from other species and human salivary amylase are inhibited, while microbial and plant glucosidases are not. The inhibition of amylase from porcine pancreas is linear up to 80% inhibition. There is no complete inhibition. By extrapolation to 100% inhibition a molar ratio of enzyme to inhibitor of 1 : 1 is found. The mechanism of inhibition is explained by a protein-protein interaction, a linkage near to the active center of the α-amylase being assumed. Both inhibitors have a similar arrangement of amino acids and a high content of aspartic acid (15.4 and 15.0 mol%, respectively) and alanine (12.8 and 12.5 mol%, respectively). They contain neither lysine nor methionine.

An α-amylase inhibitor was obtained from culture filtrates of *Streptomyces fradiae* (FERM-P 2303) by precipitation with ammonium sulfate and subsequent chromatography[54]. This preparation designated as X-2 has a molecular weight of approximately 6500 and is a carbohydrate-free acidic polypeptide with *N*-terminal aspartic acid (or asparagine).

[*] Haim = hog pancreatic α-amylase inhibitor of microbes.

11. Mechanism of Inhibition of Sucrase by Acarbose, Nojirimycin and 1-Deoxynojirimycin

The most thorough investigations into the inhibition mechanism of α-glucosidase by microbial inhibitors have so far been carried out by *Semenza et al.*[88] on the isolated sucrase-isomaltase complex from rabbit small intestine[89]. Acarbose *(5)* inhibits the sucrase activity of disaccharidase preparations from the small intestine of various species by a fully competitive mechanism[23,88,90,91]. The inhibitor constants (K_i values) listed in Table 12 range, depending on the species, between 10^{-6} and 10^{-7} mol/L. The K_i-value of component 2 *(6)* is 7.0×10^{-7} mol/L (sucrase from porcine pancreas; method: "Dixon plot").

Table 12. Sucrase inhibitor constants (K_i) of acarbose *(5)*; substrate: sucrose.

Origin of intestinal disaccharidase preparation	K_m [mol/L]	K_i [mol/L]	pH	Method	Ref.
Man	1.9×10^{-2}	1.3×10^{-6}	6	"Dixon plot"	[90, 91]
Hog	1.9×10^{-2}	2.6×10^{-7}	6.25	"Dixon plot"	[23]
Rat		$\sim 4 \times 10^{-7}$		"Dixon plot"	[91]
Rabbit [a]	8×10^{-3}	4.7×10^{-7}	6.8	"Henderson plot"	[88]

[a] K_m value according to [92, 93].

Nojirimycin *(35)* and 1-deoxynojirimycin *(36)* are also fully competitive inhibitors of sucrase. Their K_i-values (enzyme preparations from rabbit; method: "Henderson plots" ("steady state" rates)) are 1.3×10^{-7} mol/L and 3.2×10^{-8} mol/L, respectively, at pH 6.8, the optimum of sucrase activity[88]. Consequently, these four substances can be regarded as the, so far, most potent unusually tight-binding sucrase inhibitors. Their affinity for the enzyme is 5 to 6 orders of magnitude greater than that of the substrate sucrose (K_m: approximately 8.0×10^{-3} mol/L[92,93]).

According to thorough kinetic studies[88] the reaction of *(5)*, *(35)* and *(36)* with the enzyme is slow: the steady state is reached within five minutes. In view of the structure of these inhibitors and the pH-dependence of their K_i-values, an interaction with the active center of the sucrase sub-unit can be assumed which would confirm the mechanism of action of sucrase suggested by *Cogoli* and *Semenza*[92]. The individual steps of the effect of sucrase are shown schematically in Figure 19. In simplified terms we can say that in a first step (A) the substrate molecule which consists of a glucose and a fructose residue is bound by the active center of the enzyme and the glucose pyranose ring is deformed. In a second step (B and C) the glucosidic oxygen function in the 1-position is protonated (possibly by the carboxyl groups in γ-position of a glutamic acid residue in the active center of the enzyme[94]). In a third step (C) the fructose residue is detached and an oxonium/carbenium cation formed which is stabilized by a carboxylate group

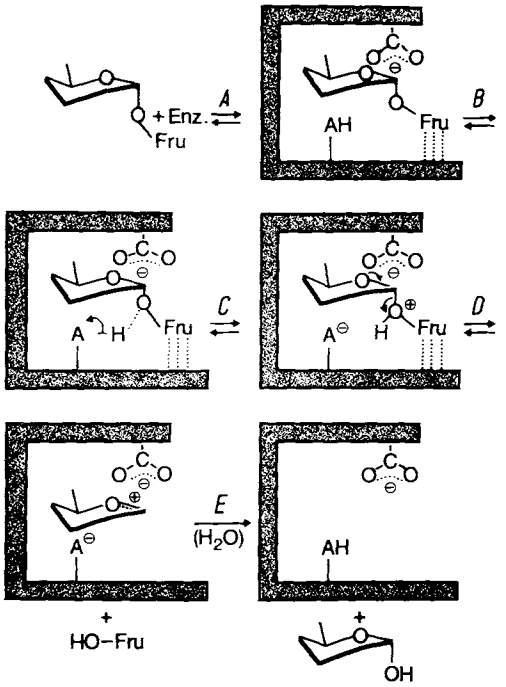


Fig. 19. Mechanism of action of enzymatic hydrolysis of sucrose by intestinal sucrase according to *Cogoli* and *Semenza* [92].

of the active center. In all probability, this is a carboxylate group in β-position of an aspartic acid residue[88,94,95]. In a last step (E) the oxonium/carbenium cation stabilized in the way described reacts with water to form D-glucopyranose—possibly *via* a covalent intermediary stage.

It is most likely that the unsaturated cyclitol unit of acarbose *(5)* interacts with the glucopyranosyl binding center of sucrase, the axially arranged nitrogen atom taking the position of the glycosyl-oxygen atom in the substrate molecule. The 4-amino-4,6-dideoxyglucose unit interacts with the so-called "aglucon" binding center. This interpretation is based on the following facts:

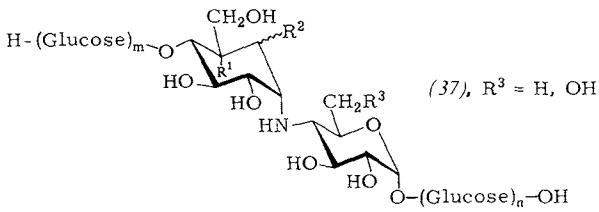
a) The unsaturated cyclitol unit has a half-chair conformation. Three of its four coplanar C atoms correspond with positions C2—C1—O—C5 in the oxonium/carbenium ion (cf. Fig. 19). However, the structural correspondence of cyclitol unit and carbenium cation is not so complete that the saturation of the double bond would lead to a drastic loss of efficacy.

b) Of greater importance for the pronounced inhibitory effect is the replacement of the glycosidic oxygen function of the substrate by the more basic NH function in the inhibitor. The pK_a value of the NH group in *(5)* is about 5[96]. This means that protonation is unlikely to occur at any of the pH values tested (5.85; 6.8; 7.45)[88]. Since this group corresponds with the glycosidic oxygen of the substrate molecule, it will probably interact with the same carboxyl group of the active center. The pK_a value of that group is about 7.3[88]. Actually, the affinity of acarbose *(5)* decreases when the carboxyl group is deprotonated (K_i at pH 6.8: 4.7×10^{-7} mol/L compared to 1.0×10^{-6} mol/L at pH 7.45)[88].

c) Also important for the high affinity for the enzyme is a marked interaction of the 4-amino-4,6-dideoxyglucose residue in *(5)* with the "aglucon" binding center, for valienamine *(16)* is a weaker inhibitor of sucrase than acarbose by 2 to 3 orders of magnitude. In addition, the α(1→4)-like linkage of cyclitol unit and amino sugar contributes to a specifity of inhibition. Consequently, acarbose inhibits neither the isomaltase sub-unit—which is closely related to the sucrase sub-unit—nor the similarly acting β-glucosidase from almonds to any appreciable degree.

All these findings are consistent with the concept of the mechanism of action of sucrase and demonstrate that the pseudosaccharide "core" of the individual members of the homologous acarbose series is essential to the inhibitory effect.

On account of the results of our own studies (see Section 5) with products of the hydrogenation of acarbose *(5)*[73] and the properties of saturated microbial inhibitors[72b] related to acarbose *(5)* described by Japanese authors, the double bond has probably only quantitative significance. According to the present state of knowledge and further references in the patent literature[68] the general structural formula *(37)* follows for these pseudo-oligosaccharidic α-glucosidase inhibitors; here $R_1 = H$, $R_2 = H$ or OH, or R_1 and R_2 form a double bond, and $R_3 = H$ or OH.



(37), R^3 = H, OH

Nojirimycin *(35)* and 1-deoxynojirimycin *(36)* have a similar molecular size and, to a certain extent, also a structure similar to D-glucose—in contrast to acarbose *(5)*. They inhibit quite a number of both α-glucopyranosidases (including isomaltase from small intestine[*], cf. Table 10) and β-glucopyranosidases[59,60,97,98]. They are likely to react with that part of the active center of sucrase which is the "glucopyranosyl subcenter", and neither with the "aglucon subcenter" nor the carboxyl group that protonates the glucosidic oxygen (pK_a approximately 7.3). Accordingly, the K_i values for both substances are practically equal at pH values 6.8 and 7.45[88]. On the basis of the data availa-

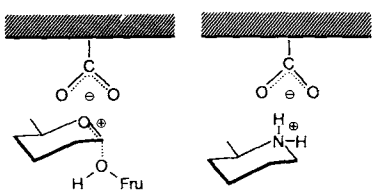


Fig. 20. Binding of the protonated 1-deoxynojirimycin molecule *(36)* to a carboxylate group of the active center of sucrase, in comparison with the stabilization of the oxonium/carbenium cation transition state of the substrate (see also Fig. 19).

[*] K_i for 1-deoxynojirimycin *(36)*: 4.8×10^{-8} mol/L at pH 6.25 (isomaltase from porcine small intestine, method: "Dixon plot").

ble the mechanism of action of nojirimycin *(35)* and deoxynojirimycin *(37)* is not as easy to explain as that of acarbose *(5)*. After a thorough discussion of all facts, including also the results obtained with other weaker inhibitors of sucrase (such as D-glucono-1,5-lactone and D-glucono-1,5-lactam), and considering hypotheses possibly applicable, *Semenza et al.*[88] arrive at the conclusion that *(35)* and *(36)* are bound to the active center in a non-protonated form and are subsequently protonated. In this case, a diagram as shown in Figure 20 could be drawn up for *(36)*.

12. Pharmacological and Clinical Aspects

For a long time dietetic rules have been a basic treatment of metabolic diseases such as diabetes mellitus, obesity or Type IV hyperlipoproteinaemia. As a rule, these regimens require a reduced intake of carbohydrates and a partition of daily meals into many small portions eaten through the day. If these instructions are followed, hyperglycaemia (excessive blood glucose values), hyperinsulinaemia (excessive blood insulin values) and hypertriglyceridaemia (increased triglyceride blood levels) can be prevented in many cases. However, in daily practice the observance of such restrictions is often hindered by unfavorable circumstances and inner resistance[45].

Carbohydrates are a main component of human food, 80–90% consisting of starch and sucrose[99]. In general more than 250 g of di- and polysaccharides—as is described in detail in Sections 2 and 3—must be enzymatically split in the intestinal tract before they can be utilized by the organism. According to the concept by *Puls*[19,20,45]—referred to in the introduction—a pharmacological interference with the intestinal carbohydrate digestion by suitable α-glucosidase inhibitors should be a feasible way to regulate and retard carbohydrate digestion, control the rate of absorption of monosaccharides and by this way influence the intermediary metabolism of the carbohydrates. As already mentioned in the introduction this concept was supported by an α-amylase inhibitor (BAY d 7791), a protein obtained from wheat germ flour[17,18], by experimental and clinico-pharmacological studies[17,19,77], which induced the search for more potent inhibitors of α-glucosidases. The pseudo-oligosaccharidic inhibitors of the general formula *(4)* proved to be particularly suitable. The results obtained with various homologues from *in vivo* experiments with rats are compared to the *in vitro* inhibitory activities against α-amylase and sucrase (Table 13, see also Table 7)[43,45,74,77].

Table 13. Inhibition of α-glucosidase activity *in vitro* and *in vivo* by acarbose *(5)* and homologues of the general formula *(4)* [45].

Component	*In vitro* 50% inhibition of 100 mU α-amylase [a] [ng]	*In vivo* ED_{50} [b] with starch [mg/kg rat]	*In vitro* 50% inhibition of 10 mU saccharase [a] [ng]	*In vivo* ED_{50} with sucrose [mg/kg rat]
2	855	1.1	560	1.0
3 (acarbose)	680	1.5	74	1.1
4	1135	1.4	455	3.3
5	4.7	1.0	700	10.0
6	2.8	0.4	700	~25.5
BAY e 4609	14.5	6.1	60000	>75.0

[a] See footnote [b], Table 7. [b] ED_{50} is the dose necessary to reduce the integrated postprandial increase in blood glucose by 50% [100].

The *in vivo* activity of the inhibitors is determined by loading tests with rats using the substrates starch and sucrose. After the administration of a defined amount of carbohydrates postprandial blood glucose concentrations are determined at various times, plotted and integrated.

The blood glucose concentrations of rats treated with physiological saline are used as a baseline. The *in vivo* activity is expressed numerically as ED_{50}. Comparison of the data for the *in vivo* activity in the sucrose loading tests for the first five members of the homologous series and the high-molecular preparation BAY e 4609 (given in Table 13) with the *in vitro* data of sucrase inhibitors shows that *in vitro* and *in vivo* activities are very much parallel—with the exception of component 2 *(6)*, the values of which cannot be satisfactorily explained at present.

Acarbose *(5)* stands out against the other compounds as the most effective inhibitor both *in vivo* and *in vitro*. It is striking that—according to Table 13—components 2–6 inhibit the degradation of starch *in vivo* to a very similar degree while there are considerable differences in the inhibition of α-amylases *in vitro* (Fig. 19 and Table 7). We can conclude from this that the *in vitro* inhibition test, using pure α-amylase as the enzyme, does not reflect the real course of the intestinal degradation of starch *in vivo*. As already mentioned in Section 3, the intestinal degradation of starch is a complex process involving a great number of enzymes. Table 7, according to which components 2–5 and especially acarbose *(5)* inhibit maltase, "dextrinase" and glucoamylase to a considerable degree, gives an idea of feasible mechanisms of the *in vivo* effects. If it were possible to exactly define the involvement of the various enzymatic activities in the *in vivo* degradation of starch to glucose, one might be able to explain the mechanism of *in vivo* action of acarbose and its homologues. It can be seen from Table 13 that the ED_{50} for acarbose *(5)* in the starch loading test is 1.5 mg/kg in the rat, thus only slightly higher than the ED_{50} determined in the sucrose loading test. This finding contributed to our selecting acarbose for clinical trials and thus for practical use. On account of its broader spectrum of inhibitory action *in vivo* acarbose proved to be clearly superior to the α-amylase inhibitor BAY e 4609 which was also subjected to in-depth pharmacological[77] and clinical studies[78].

Acarbose does not only produce a dose-dependent inhibition of the postprandial rise in blood sugar values in rats in starch and/or sucrose loading tests, but also inhibits the reactive postprandial hyperinsulinaemia[45,100]. Therapy-related clinico-pharmacological studies on healthy test persons have yielded similar results as the rat experiments described above[45,75,100].

Moreover, acarbose was found to reduce the weight of genetically obese "Zucker" rats that had received a sucrose-containing diet—in a dose-dependent manner—; this can be attributed to a reduced food intake by the animals treated. These animals also had significantly lower serum levels of triglycerides and free fatty acids. The reduction of carbohydrate-induced hyperlipoproteinaemia is considered to be due to a decrease in the excessive secre-

tion of high-triglyceride lipoproteins, the so-called "VLDL fraction", from the liver. Analyses of the animals' trunks (carcasses) revealed a reduced accumulation of fat in the body but unchanged protein content[45, 100] in comparison with controls.

Acarbose *(5)* is the clinically most thoroughly tested compound especially in the indication diabetes mellitus. The results of the numerous large-scale studied carried out so far can be summarized as follows[75, 76, 101]:

Acarbose clearly improves the metabolic condition of insulin-dependent diabetics. Blood glucose levels are lowered so that the dose of insulin can be reduced. However, if there is a deficiency of insulin, acarbose cannot replace insulin. In non-insulin-dependent diabetics the improvement of the metabolic condition is the greater the longer acarbose is administered. On account of its mechanism of action, acarbose may produce transient intestinal symptoms which, however, are usually tolerated by the patient. These symptoms are reduced, *i.e.* tolerability improves, especially when the drug is taken over a prolonged period. Haematological and clinical-biochemical studies have furnished evidence for the preparation's objectively very good tolerability. Acarbose—which has not yet been put on the market—can thus be regarded as a new active principle in the treatment of diabetes mellitus. The efficacy of this preparation in hyperlipoproteinaemia and adiposity is under clinical investigation.

In order to establish additional indications for microbial α-glucosidase inhibitors investigations were performed with test persons into the inhibition of oral starch degradation as effected mainly by salivary amylase[102]. It was shown that the addition of the α-amylase inhibitor BAY e 4609 to starch results in a reduction of oral starch degradation. After administration of starch, telemetry can be used to follow the rapid drop of pH which is caused by the bacteria within the "plaques" on the teeth. A smaller pH drop was observed in the presence of the amylase inhibitor. This effect was amplified by the combination of BAY e 4609 with acarbose. These results support the idea that microbial α-glucosidase inhibitors might reduce the cariogenic potential of starch during its degradation in the oral cavity[102].

We have seen by the example of acarbose and the other α-glucosidase inhibitors discussed in this article that new active substances from the far-from-exhausted reservoir of microbial secondary metabolites can be discovered by means of practice-related screening tests.

We are indebted to Prof. Dr. G. Semenza, Biochemical Laboratory of ETH Zürich, for the extremely valuable discussions of the mechanism of action of the α-glucosidase inhibitors and for making his manuscript[88], *still unpublished when the present paper was submitted, available to us. We also express our gratitude to Dr. J. J. Marshall, Miles Laboratories Inc., Elkhart, Indiana (USA) for permitting the use of the data listed in Tables 8 and 9 which are part of a manuscript*[87] *still unpublished when this paper was written.*

Received: January 19, 1981 [A 372 IE]
German version: Angew. Chem. *93*, 738 (1981)

[1] *H. Fritz, H. Tschesche:* Proceedings of the International Research Conference on Proteinase Inhibitors, de Gruyter, Berlin 1971.
[2] *H. Fritz, H. Tschesche, L. J. Greene, E. Truscheit:* Bayer-Symposium V, Proteinase Inhibitors, Springer, Berlin 1974.
[3] Review: *H. Tschesche,* Angew. Chem. *86*, 21 (1974); Angew. Chem. Int. Ed. Engl. *13*, 10 (1974).
[4] Review: *M. Laskowski, Jr., I. Kato,* Annu. Rev. Biochem. *49*, 593 (1980).
[5] *C. Fermi, L. Pernossi,* Z. Hyg. Infektionskr. *18*, 83 (1894).
[6] Summary by *N. Heimburger, H. Haupt, H. G. Schwick* in [1], p. 1.
[7] *T. Chrzaszcz, J. Janicki,* Biochem. Z. *260*, 354 (1933); *264*, 193 (1933).
[8] *E. Kneen, R. M. Sandstedt,* J. Am. Chem. Soc. *65*, 1247 (1943).
[9] *E. Kneen, R. M. Sandstedt,* Arch. Biochem. *9*, 235 (1946).
[10] *D. E. Bowman,* Science *102*, 358 (1945).
[11] *A. Hernandez, W. G. Jaffé,* Acta Cient. Venez. *19*, 183 (1968); *W. G. Jaffé, R. Moreno, V. Wallis,* Nutr. Rep. Int. *7*, 169 (1973).
[12] *J. J. Marshall, C. M. Lauda,* J. Biol. Chem. *250*, 8030 (1975).
[13] *J. R. Powers, J. R. Whitaker,* J. Food Biochem. *1*, 217, 239 (1977).
[14] *K.-H. Pick, G. Wöber,* Z. Physiol. Chem. *359*, 1371, 1379 (1978); Prep. Biochem. *9*, 293 (1979).
[15] *M. N. Rao, K. S. Shurpalekar, O. E. Sundaravalli,* Indian J. Biochem. *4*, 185 (1967); *7*, 241 (1970).
[16] Reviews: a) *J. J. Marshall:* Physiological Effects of Food Carbohydrates in ACS Symp. Ser., Vol. 15, Am. Chem. Soc., Washington 1975, p. 244ff.; b) *V. Buonocore, T. Petrucci, V. Silano,* Phytochemistry *16*, 811 (1977); c) Proc. 3. Int. Congr. Obesity, Satellite Symp. No. 7, Siena (Italien), October 13.–15, 1980; Regulators of Intestinal Absorption in Obesity, Diabetes and Nutrition; d) *V. Buonocore, E. Poerio, V. Silano* in [16c], Vol. 1, p. 87; Abstract see: La Clinica Dietologica *7*, 429 (1980). Recent works: e) *P. E. Granum, J. R. Whitaker,* J. Food Biochem. *1*, 385 (1977); f) *P. E. Granum, ibid. 2*, 103 (1978); g) *M. Richardson, N. Kashlan* in [16c], Vol. 1, p. 103; h) *C. M. O'Connor, M. D. O'Donnell, K. F. McGeeney* in [16c], Vol. 2, p. 119; Abstract see: La Clinica Dietologica *7*, 443 (1980); i) *C. M. O'Connor, K. F. McGeeney,* Biochim. Biophys. Acta *658*, 387, 397 (1981).
[17] *D. D. Schmidt, W. Puls,* DOS 2003934 (1971) (German priority January 26, 1970), Bayer AG.
[18] *E. Truscheit, D. D. Schmidt, A. Arens, H. Lange, W. Wingender* in [16c], Vol. 2, p. 157; Abstract see: La Clinica Dietologica *7*, 451 (1980).
[19] *W. Puls, U. Keup,* Diabetologia *9*, 97 (1973).
[20] *W. Puls,* Habilitationsschrift, Universität Düsseldorf 1980.
[21] *W. Frommer, W. Puls, D. Schäfer, D. D. Schmidt,* DOS 2064092 (1972) (German priority December 28, 1970), Bayer AG.
[22] *W. Frommer, B. Junge, U. Keup, L. Müller, W. Puls, D. D. Schmidt,* DOS 2347782 (1975) (German priority September 22, 1973), Bayer AG.
[23] *D. D. Schmidt, W. Frommer, B. Junge, L. Müller, W. Wingender, E. Truscheit, D. Schäfer,* Naturwissenschaften *64*, 535 (1977).
[24] *H. Umezawa:* Enzyme Inhibitors of Microbial Origin, University Park Press, Baltimore 1972.
[25] *W. Frommer, B. Junge, L. Müller, D. D. Schmidt, E. Truscheit,* Planta Med. (J. Med. Plant Res.) *35*, 195 (1979).
[26] *W. Wingender* in [2], p. 548; *T. Aoyagi, H. Umezawa* in *E. Reich, D. B. Rifkin, E. Shaw:* Proteases and Biological Control, Cold Spring Harbor Conf. Cell Proliferation *2*, 429 (1975); *H. Umezawa, T. Takita, T. Shiba:* Bioactive Peptides Produced by Microorganisms, Wiley, New York 1978; *T. Aoyagi, H. Umezawa* in *Lj. Vitale, V. Simeon:* Industrial and Clinical Enzymology, FEBS Symp. *61*, 89 (1980).
[27] *W. J. Whelan,* Biochem. J. *122*, 609 (1971).
[28] *D. J. Manners,* Essays Biochem. *10*, 37 (1974).
[29] *J. J. Marshall,* Adv. Carbohydr. Chem. Biochem. *30*, 257 (1974).
[30] *Z. Gunja-Smith, J. J. Marshall, C. Mercier, E. E. Smith, W. J. Whelan,* FEBS Lett. *12*, 101 (1970).
[31] *J. W. Mayo, D. M. Carlson,* Arch. Biochem. Biophys. *163*, 498 (1974).
[32] *P. Cozzone, G. Marchis-Mouren,* Biochim. Biophys. Acta *257*, 222 (1972).
[33] *P. Bernfeld,* Methods Enzymol. *1*, 149 (1955).
[34] *R. Ruyssen, A. Lauwers:* Pharmaceutical Enzymes – Properties and Assay Methods, p. 66, Story-Scientia, Gent 1978.
[35] *K. Fossum, J. R. Whitaker,* J. Nutr. *104*, 930 (1974).
[36] *J. J. Marshall, C. M. Lauda,* Stärke *27*, 274 (1975).
[37] *G. Semenza,* Enzymes Biol. Membr. *3* (Membrane Transport), 349 (1976).
[38] *J. K. Kelly, D. H. Alpers,* Biochim. Biophys. Acta *315*, 113 (1973).
[39] *K. A. Conklin, K. M. Yamashiro, G. M. Gray,* J. Biol. Chem. *250*, 5735 (1975).
[40] *J. J. Marshall, C. M. Sturgeon, W. J. Whelan,* Anal. Biochem. *82*, 435 (1977).
[41] *G. Semenza,* Carbohydr. Metab. Its Disord., Vol. III, Academic Press, London 1981, in press.
[42] *A. Dahlqvist,* Enzymol. Biol. Clin. *11*, 52 (1970).

[43] a) *U. Brodbeck:* Enzyme Inhibitors (Proc. Meet., Basel, March 20–21, 1980), Verlag Chemie, Weinheim 1980; b) *L. Müller, B. Junge, W. Frommer, D. D. Schmidt, E. Truscheit* in [43a], p. 106.
[44] *D. D. Schmidt, W. Frommer, L. Müller, E. Truscheit,* Naturwissenschaften *66*, 584 (1979).
[45] *W. Puls, U. Keup, H. P. Krause, L. Müller, D. D. Schmidt, G. Thomas, E. Truscheit,* Front. Horm. Res. *7*, 235 (1980).
[46] *D. D. Schmidt, W. Frommer, B. Junge, L. Müller, E. Truscheit, W. Wingender* in [16c], Vol. 1, p. 203; Abstract see: La Clinica Dietologica *7*, 449 (1980).
[47] *S. Ueda, Y. Koba,* US-Pat. 3806421 (1974) (Jap. priority May 31, 1971), Hayashibara Biochemical Laboratories; Agric. Biol. Chem. *37*, 2025 (1973).
[48] *Y. Koba, M. Najima, S. Ueda,* Agric. Biol. Chem. *40*, 1167 (1976).
[49] *S. Ueda, Y. Koba, H. Chaen,* Carbohydr. Res. *61*, 253 (1978).
[50] *H. Chaen, S. Ueda,* Agric. Biol. Chem. *43*, 2265 (1979).
[51] *S. Murao,* US-Pat. 4010258 (1977) (Jap. priority March 15, 1974), Ajinomoto Co., Inc. und Eisai Co., Ltd.; *S. Murao, K. Ohyama,* Agric. Biol. Chem. *39*, 2271 (1975).
[52] *S. Murao, K. Ohyama, S. Ogura,* Agric. Biol. Chem. *41*, 919 (1977); *K. Ohyama, S. Murao,* ibid. *41*, 2221 (1977).
[53] *S. Murao, K. Ohyama,* Agric. Biol. Chem. *43*, 679 (1979).
[54] *H. Goto, T. Inukei, M. Amano,* Japanische Offenlegungsschrift 7577594 (1975) (Jap. priority November 20, 1973), Amano Pharmaceutical Co., Ltd.
[55] *W. Frommer, L. Müller, D. D. Schmidt, W. Puls, H. P. Krause, U. Heber,* DOS 2658563 (1978) (German priority December 23, 1976).
[56] *S. Inouye, T. Tsuruoka, T. Niida,* J. Antibiot. *19*, 288 (1966).
[57] *H. Paulsen, I. Sangster, K. Heyns,* Chem. Ber. *100*, 802 (1967).
[58] *S. Inouye, T. Tsuruoka, T. Ito, T. Niida,* Tetrahedron *24*, 2125 (1968).
[59] *T. Niwa, S. Inouye, T. Tsuruoka, Y. Koaze, T. Niida,* Agric. Biol. Chem. *34*, 966 (1970).
[60] *E. T. Reese, F. W. Parrish, M. Ettlinger,* Carbohydr. Res. *18*, 381 (1971).
[61] *H. Murai, K. Ohata, H. Enomoto, Y. Yoshikuni, T. Kono, M. Yagi,* DOS 2656602 (1977) (Jap. priority December 29, 1975), Nippon Shinyaku Co., Ltd.
[62] *S. Matsumura, H. Enomoto, Y. Aoyagi, Y. Ezure, Y. Yoshikuni, M. Yagi,* DOS 2850467 (1979) (Jap. priority November 21, 1977), Nippon Shinyaku Co., Ltd.
[63] *S. Murao, S. Miyata,* Agric. Biol. Chem. *44*, 219 (1980).
[64] *K. Ueda, S. Gochyo,* Japanische Offenlegungsschrift 51-54990 (1976) (Jap. priority November 5, 1974), Ajinomoto Co., Ltd.
[65] *A. Belloc, J. Florent, J. Lunel, D. Mancy, J.-C. Palla,* DOS 2702417 (1977) (French priority January 22, 1976), Rhône-Poulenc Industries.
[66] *V. Oeding, W. Pfaff, L. Vértesy, H.-L. Weidemüller,* DOS 2701890 (1978) (German priority January 19, 1977), Hoechst AG.
[67] a) *L. Vértesy, V. Oeding, R. Bender, G. Nesemann, D. Sukatsch, K. Zepf* in [16c], Vol. 2, p. 269; Abstract see: La Clinica Dietologica *7*, 452 (1980); b) *H. Aschauer, L. Vértesy, G. Braunitzer,* Z. Physiol. Chem. *362*, 465 (1981).
[68] *M. Otani, T. Saito, S. Satoi, J. Mizoguchi, N. Muto,* DOS 2855409 (1979) (Jap. priority December 28, 1977), Toyo Jozo Co., Ltd.
[69] *K. Kangouri, S. Namiki, T. Nagate, K. Sugita, S. Omura,* US-Pat. 4197292 (1980) (Jap. priority February 10, 1978), Taisho Pharmaceutical Co.
[70] *Y. Suhara, K. Yokose, K. Watanabe, K. Ogawa,* DOS 2905649 (1979) (Brit. priority February 14, 1978), Hoffmann-La Roche und Co. AG.
[71] *S. Murao, A. Goto, Y. Matsui, K. Ohyama,* Agric. Biol. Chem. *44*, 1679 (1980).
[72] a) *S. Murao, A. Goto, M. Arai,* Agric. Biol. Chem. *44*, 1683 (1980); b) *S. Omoto, J. Itoh, T. Niwa, T. Shomura, S. Niizato, S. Inouye,* DOS 3035193 A1 (1981) (Jap. priority September 19, 1979), Meiji Seika Kaisha Ltd.
[73] a) *B. Junge, H. Böshagen, J. Stoltefuß, L. Müller* in [43a], p. 123; b) *B. Junge, H. Böshagen, F. Heiker, L. Müller, J. Stoltefuss:* Lecture, Fall Meet. Am. Chem. Soc., New York 1981.
[74] *W. Puls, U. Keup, H. P. Krause, G. Thomas* in [16c], Vol. 1, p. 231; Abstract see: La Clinica Dietologica *7*, 458 (1980).
[75] *K. Schöffling, I. Hillebrand, P. Berchtold,* Front. Horm. Res. *7*, 248 (1980).
[76] *P. Berchtold, I. Hillebrand, K. Boehme, R. Aubell, L. Nagel, H. Schulz* in [16c], Vol. 1, p. 261; Abstract siehe: La Clinica Dietologica *7*, 426 (1980).
[77] *W. Puls, U. Keup, H. P. Krause, K. O'Dea, R. Sitt* in [16c], Vol. 1, p. 153; Abstract see: La Clinica Dietologica *7*, 458 (1980).
[78] *P. Berchtold, N. H. Kiesselbach* in [16c], Vol. 1, p. 181; Abstract see: La Clinica Dietologica *7*, 426 (1980).
[79] *W. Frommer, B. Junge, U. Keup, L. Müller, W. Puls, D. D. Schmidt,* DOS 2347798 (1975) (German priority September 22, 1973), Bayer AG.
[80] *W. Frommer, W. Puls, D. D. Schmidt,* DOS 2209832 (1973) (German priority March 1, 1972), Bayer AG.
[81] *S. Horii, T. Iwasa, E. Mizuta, Y. Kameda,* J. Antibiot. *24*, 59 (1971).
[82] Revidierte Struktur von Validamycin A: *T. Suami, S. Ogawa, N. Chida,* J. Antibiot. *33*, 98 (1980).
[83] *S. Horii, Y. Kameda,* J. Chem. Soc. Chem. Commun. *1972*, 747.
[84] a) *Y. Kameda, S. Horii,* J. Chem. Soc. Chem. Commun. *1972*, 746; b) *Y. Kameda, N. Asano, M. Yoshikawa, K. Matsui,* J. Antibiot. *33*, 1575 (1980).
[85] *W. Frommer, W. Puls, D. D. Schmidt,* DOS 2209833 (1973) (German priority March 1, 1972), Bayer AG.
[86] *W. Frommer, W. Puls, D. D. Schmidt,* DOS 2209834 (1973) (German priority March 1, 1972), Bayer AG.
[87] *H. Hidaka, T. Takaya, J. J. Marshall,* J. Jpn. Starch Sci. *27*, 114 (1980).
[88] *G. Hanozet, H.-P. Pircher, P. Vann, B. Oesch, G. Semenza,* J. Biol. Chem. *256*, 3703 (1981).
[89] *H. Sigrist, P. Ronner, G. Semenza,* Biochim. Biophys. Acta *406*, 433 (1975).
[90] *W. F. Caspary,* Lancet *1978*, 1231.
[91] *W. F. Caspary, S. Graf,* Res. Exp. Med. *175*, 1 (1979).
[92] *A. Cogoli, G. Semenza,* J. Biol. Chem. *250*, 7802 (1975).
[93] *G. Semenza, A.-K. von Balthazar,* Eur. J. Biochem. *41*, 149 (1974).
[94] *H. Braun, A. Cogoli, G. Semenza,* Eur. J. Biochem. *52*, 475 (1975).
[95] *A. Quaroni, E. Gershon, G. Semenza,* J. Biol. Chem. *249*, 6424 (1974).
[96] Nach Untersuchungen von *W. Gau,* Bayer AG, Wuppertal, persönliche Mitteilung.
[97] *A. K. Grover, R. J. Cusley,* Biochim. Biophys. Acta *482*, 109 (1977).
[98] *G. Legler, K.-R. Roeser, H.-K. Illig,* Eur. J. Biochem. *101*, 85 (1979).
[99] Ernährungsbericht 1976, Deutsche Gesellschaft für Ernährung, Frankfurt 1976.
[100] *W. Puls, U. Keup, H. P. Krause, G. Thomas, F. Hoffmeister,* Naturwissenschaften *64*, 536 (1977).
[101] *I. Hillebrand, P. Berchtold* in [43a], p. 153.
[102] *J. E. Mörmann, H. R. Mühlemann,* Lebensmittelwiss. Technol. *14*, 166 (1981).