Translated from *Uspekhi Khimii*, 50, 590-605 (1981) U.D.C. 547.466.07

Aspartame and Its Analogues

L.A.Pavlova, T.V.Komarova, Yu.A.Davidovich, and S.V.Rogozhin

The results of studies on the biochemistry of the sweet taste are briefly reviewed. The methods of synthesis of "aspartame"—a sweet dipeptide—are considered, its structural analogues are described, and quantitative estimates are made of the degree of sweetness relative to sucrose. Attention is concentrated mainly on problems of the relation between the structure of the substance and its taste in the series of aspartyl derivatives.
The bibliography includes 118 references.

CONTENTS

I. Introduction 316
II. Certain aspects of the biochemistry of the sweet taste 316
III. Methods of synthesis of aspartame 317
IV. Relation between the structure of a substance and its taste in the series of aspartame analogues 318

I. INTRODUCTION

After the accidental discovery by Schlatter and coworkers[1] in 1969 of the sweet taste of the methyl ester of α-L-aspartyl-L-phenylalanine, numerous studies devoted to the synthesis and applications of "aspartame" (this was the name given to this sweet preparation, first manufactured on an industrial scale by the American G.D.Searle Company[2]) have been published. The study of the structural analogues of aspartame from the standpoint of their taste merits no less attention. Studies on these lines have been prosecuted on a wide scale since 1969 until today and have made a considerable contribution to the theory of the sweet taste. The problem of the relation between structure and taste, the solution of which will permit a specific search for new intensely sweet compounds, its particularly urgent[3].

The increased interest in sweet peptides is justified also from the practical point of view. The need for synthetic flavours designed to replace sucrose continuously increases[4,5]. Many representatives of the series of dipeptide esters are superior to sucrose as regards the intensity of the taste by factors of hundreds and thousands. Their application would make it possible to reduce sharply the calorie content in view of the small amount of substance required. The assimilation of such products is unrelated to insulin. By virtue of these properties, sweet peptides (together with other low-calorie natural and synthetic sweet substances) can be used in low calorie diets and are recommended primarily for patients suffering from obesity and sugar diabetes[4-9].

Being indifferent to microorganisms, sweet dipeptides such as aspartame are particularly convenient for pharmaceutical use (for example for the sweetening of medicines, which is extremely important for the treatment of children[10-12], particularly since aspartame is safe for both children and adults[13]). From the medical point of view, aspartame has yet another important advantage over sucrose: it does not stimulate the development of dental caries[5].

When aspartame is used in combination with sucrose, glucose, cyclamate, or saccharin, a synergistic effect is produced, as a result of which the consumption coefficients of both mixture components are reduced[5]. Furthermore, it has been observed that the addition of a small amount (2–3 wt.%) of aspartame or some of its analogues to saccharin fully masks its unpleasant flavour[12].

Among the most widely used synthetic sweeteners employed abroad at the present time (particularly in the USA), aspartame is superior as regards its taste[4,5]. The metabolism and potential toxicity of aspartame have been thoroughly investigated[14,15]. This preparation has withstood numerous tests for toxicity and carcinogenic properties and is contraindicated only for patients suffering from phenylketonuria[2,5]. It has been noted that aspartame influences the pH of human whole saliva and tends to alter the concentration of lactic acid in it[16].

Aspartame is convenient for use in a wide variety of dietary compositions[5]. The only limitation is its lability with respect to hydrolysis in strongly acidic and neutral-alkaline media, particularly on heat treatment[5,17]. Methods have been proposed for the modification of labile dipeptide esters which eliminate this disadvantage. One of the ways of reducing the hydrolytic lability of sweetening agents consists in binding the molecules responsible for the sweet taste to macromolecules, for example, dextran[18,19] or the soya-bean protein extract[20]. Such molecular aggregates are obtained when aqueous solutions of both components are dried together (in the required proportions) by spraying or sublimation. It is then important to find the optimum size of the aggregate, since an increase of molecular size hinders the attainment of the required contact with the receptor and can lead to an appreciable decrease of the degree of sweetness. Another way is to synthesise compounds with a structure similar to that of aspartame but without a tendency towards hydrolysis and the formation of diozopiperazine derivatives in aqueous solutions[21]. (These compounds are considered in Section 4 of the present review.)

Thus the importance of synthetic peptide flavours is evident. The problem arises of the development of the most rational and economical syntheses of the best examples of the compounds under consideration, which would make them sufficiently readily available at least for therapeutic purposes.

II. CERTAIN ASPECTS OF THE BIOCHEMISTRY OF THE SWEET TASTE

Before considering the methods of synthesis and the structure–taste relations for the enormous number of compounds obtained on the basis of the aminoacids, it is useful to describe briefly the problems of the theory of the sweet taste.

The taste reaction is a complex phenomenon which has been inadequately investigated. The perception of taste arises as a result of the interaction of the molecules of the substance stimulating the taste (tastant) with the corresponding chemoreceptor. The human sensor systems incorporate several types of the so-called taste buds, i.e. sensitive sections of the tongue. It has been established[22] that the receptors responsible for the sweet taste are located

mainly in the front part of the tongue in mushroom-shaped buds. The buds contain taste cells, in the membranes of which chemoreceptors are believed to be distributed[23,24].

The protein forming complexes with sugars and apparently behaving as a receptor responding only to sweet substances[25–29] has been isolated from the epithelial tissues of animal tongues and was subsequently investigated. The primary chemoreception processes were investigated at the molecular level for the first time by Beidler[30–32], who established, in particular, that physical processes of the type of adsorption underlie the interaction of the tastant with the active centres of the plasmatic membrane of the tast cell, this hypothesis was subsequently confirmed[33].

The wide variety of sweet substances having completely different compositions, constitutions, and geometric structures interfered for a long time with the discovery of the principal distinctive features common to all sweet compounds and enabling the receptor to recognise them. A specific common feature of sweet molecules was discovered by Shallenberger and Acree[34], who specified the concept of contact between the tastant and the receptor, having established that such contact is achieved via hydrogen bonds formed within specific sections of the molecule of the tastant and the active centres of the receptor. They claim[34] that a sweet substance must have two polar functional groups, one of which behaves as a proton donor and the other as a proton acceptor; they designated them by AH and B. In order to achieve two-point binding of the molecule to the receptor via hydrogen bonds, the distance between the atoms or groups of atoms A and B in the molecule must correspond to the distance between the active centres of the receptor. It has been established[34] that it amounts to ~3 Å.

Although this theory is in fact imperfect, it is nevertheless valid in relation to different classes of compounds with a sweet taste. The mechanism of the interaction of the receptor–sweetener pair was refined on the basis of data obtained in the study of the taste of aminoacid stereoisomers[35–39]. The fact that many D-aminoacids are sweet, while their L-isomers are tasteless or bitter indicates the stereospecificity of the chemoreceptor and leads to the conclusion that there exists a third contact between the tastant and the receptor. By calculating the interaction energy in the tastant–receptor model system, it was shown[40] that this type of interaction represents hydrophobic binding. Consequently, apart from the AHB polar system, the tastant molecule should contain a hydrophobic region (it will be designated by X) oriented appropriately[41].

The degree of hydrophobic binding to the receptor apparently determines the intensity of the taste. Deutsch and Hansch[42] and Holthae and Kier[41] demonstrated the existence of this correlation in relation to alkyl-substituted 2-amino-4-nitrobenzenes. It will be shown below, in relation to specific examples, how great is the influence of the hydrophobic properties and the geometric form of the substituents in the side chain in the series of aspartame analogues on the intensity of the taste of these compounds. It has been suggested that the third binding region X in the series of dipeptide esters is located at the centre of a branched substituent in the side chain; for example, this is the centre of the benzene ring in the case of aspartame[43].

It has been suggested[44–46] that the more exact the correspondence of the surface features of the tastant molecule and the "working" surface of the receptor, the more readily is the required contact between them attained and the stronger the perception of the taste. The external form of the "sweet unit" can thus serve approximately as a model of the active receptor surface[46]. One of the preferred conformers of aspartame, which is capable of binding to the receptor without steric hindrance, was chosen as a model[44–46] (Fig. 1a). The authors claim that this model surface of the receptor is consistent with the shape of the molecules of sweet substances of different types, which can be useful for the prediction of the taste of new structural analogues of aspartame and other compounds.

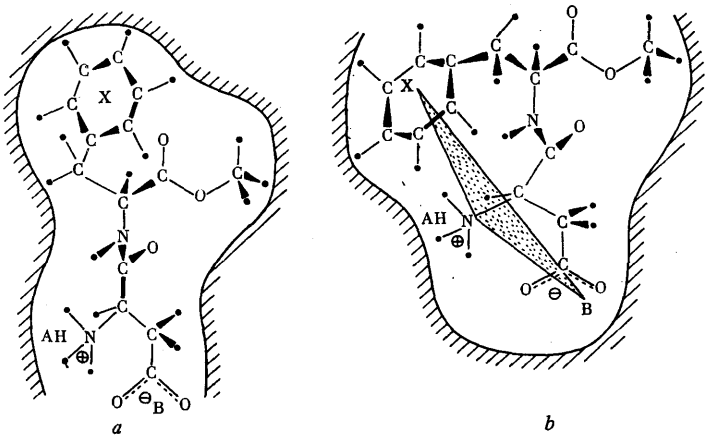


Figure 1. The suggested conformation of the aspartame molecule and of the active site of the taste receptor according to the data of Lelj et al.[44] (a) and Heijden et al.[43] (b).

Another aspartame conformer was selected as a model of the receptor by Heijden et al.[43] (Fig. 1b). According to the authors[43], only for this structure of the sweet dipeptide can the identity of the taste be influenced by the dimensions and geometrical shape of the side chain and of the terminal ester group, which ensure the maximum coupling with the receptor when the correspondence between the surfaces is satisfactory; furthermore, the structural data for this conformer agree best with the calculated A–B–X distances. Yet another model of the hydrophobically binding part of the receptor, obtained on the basis of structural data for the sweetest diesters of α-aspartylaminomalonic acid, has been proposed[47]. The contradictions between different authors as regards the topology of the taste receptor are due to the fact that their conclusions are based on calculations for individual static conformations of aspartame (or other sweet compounds) without taking into account the structural changes in the course of the interaction with the receptor. More accurate data can be obtained by the direct investigation of receptor–dipeptide ester complexes.

Despite the available data[24,40,48–53], the mechanism of the interaction of the tastant with the receptor required further thorough investigation. One cannot rule out the possibility that certain enzymes are involved in the perception of taste[24,54].

III. METHODS OF SYNTHESIS OF ASPARTAME

The methyl ester of α-L-aspartyl-L-phenylalanine has an intense sweet taste, being 150–180 times sweeter than sucrose. One of its component aminoacids (phenylalanine) is an unsubstituted natural aminoacid.

Numerous studies have been carried out on the methods of synthesis of aspartame. The most attractive procedure in view of its ease and the low cost of the starting material is

the method based on the aminolysis of the inner anhydride of aspartic acid. The anhydride of aspartic acid with the amino-group protected by groups usually employed in peptide synthesis (benzoxycarbonyl, t-butoxycarbonyl, o-nitrophenylsulphenyl, formyl, etc.[55–60]) and the anhydride in the form of different salts[61–66] are used equally widely. The aminolysis is carried out in organic solvents, aqueous organic mixtures, and water (at a specific pH)[59].

The aminolysis of the salts of aspartic acid anhydride can be carried out selectively with the preferential formation of the α-isomer by reducing the temperature, by employing an excess of the amino-component, and by introducing various additives into the reaction mixture[61]. These additives can be (1) acids (acetic acid[61], carbon dioxide[62–64], and orthophosphoric acid[65]); (2) mixtures of weak acids and lower aliphatic alochols[62]; (3) mixtures of strong acids and alcohols[66,67]. Aqueous solutions of inorganic bases have been used for the same purpose as additives in the aminolysis of the *N*-benzoxycarbonylanhydride of aspartic acid[68].

The above group of methods is characterised by general advantages and disadvantages: on the one hand, there is the evident simplicity and low cost of the method of synthesis and, on the other hand, the problem of freeing the main product from undesirable (in the given synthetic scheme) impurities, primarily the corresponding β-isomer, which is difficult to separate from the main product and has bitter taste[69–71].

The problem of separating the α- and β-isomers having opposed tastes is solved in different ways, which are based on the following factors: (1) on the different solubilities of their salts with strong inorganic acids[58,72,73] or with aromatic acids[74] (the α-isomer salts are usually less soluble in aqueous and aqueous organic media than the corresponding salts of the β-isomer); (2) the reduced reactivity of the β-isomer in the reaction with ketones. On treatment with acetone, the α-isomers of aspartame and some of its analogues readily form the corresponding 4-imidazolidinones, which are readily soluble in acetone; the β-isomers, which do not react, remain insoluble[75,76]. The latter method suffers from the following disadvantage: in order to isolate aspartame in a free form, the fairly stable 4-imidazolidinone must be hydrolysed at an elevated temperature, whereupon there is a possibility[77] of the partial decomposition of the labile aspartame.

A novel method has been proposed for synthesising aspartame, which has been recommended as an industrial procedure; it is based on the interaction of the *N*-protected aspartic acid with the amino-component in the form of an ester of an *N*-carbonylaminoacid[78]. The main disadvantage of this method is the necessity to work with phosgene at elevated temperatures (this imposes certain requirements on the apparatus) and also the inevitable formation of the β-isomer as a side product.

Aspartame has been obtained[79] by the interaction of the *N*-protected aspartic acid with formaldehyde and subsequent aminolysis of the oxazolidinone, which, in view of its inadequate reactivity, reacts with the amino-component only at an elevated temperature (50 °C). A method has been developed for the synthesis of sweet α-aspartyl derivatives using the *N*-carboxyanhydride of a β-*O*-protected derivative of aspartic acid[80]. A bottleneck in this procedure is the stage involving the aminolysis of the *N*-carboxyanhydride, which is achieved in the pH range 9–12 and under these conditions there is a possibility of the hydrolysis of the ester group.

Aspartame has also been synthesised[81] by a non-traditional method from the dichloride of maleic acid. It is important to note that this yields only the DL–L-form. It has been suggested that aspartame be obtained from (*S*)–4-vinylazetidin-2-one,[82] but the method is inconvenient owing to the instability of the intermediates.

Naturally the classical methods of peptide synthesis (the carbodi-imide, mixed anhydride, or activated esteer methods) have also been used to obtain aspartame and its analogues[83–85]. Their advantages and disadvantages are well known[86]. A version of the synthesis of aspartame using the convenient condensing agent trifluoroacetoxysuccinimide has been described[87]. This reagent ensures the rapid and complete conversion of the *N*,β-*O*-disubstituted carboxy-component into the corresponding α-*N*-hydroxysuccinimide ester, which readily undergoes aminolysis.

Modified methods for the removal of the protecting groups in the course of the synthesis of free dipeptide esters with a sweet taste have been put forward[88–90]. It has been reported[91] that the manufacture of aspartame by an enzymatic method from racemic aminoacids, which are the most convenient and economical starting material, has been stablished in Japan[91].

Table 1. The relative degrees of sweetness (RS) of aspartame, its isomers, and analogues[1].

Compound	RS*
Sweet	
α-(L)-Asp-(L)-Phe—OMe	100—150
α-(L)-Asp-(L)-Met—OMe	100
α-(L)-Asp-(L)-Tyr—OMe	10
α-(L)-Asp-(L)-Phe—OEt**	10
α-(L)-Asp-(L)-Phe—OPr-n**	1
α-(L)-Asp-(L)-Phe—OPr-iso**	1
α-(L)-Asp-(L)-Phe—OBu-t**	1
Tasteless	
α-(L)-Asp-(L)-Phe	0
α-(L)-Asp-(L)-Phe—NH_2	0
α-(L)-Asp-(L)-Phe (NH₂)	0
Bitter	
α-(L)-Asp-(L)-Phe (OMe)	
β-(L)-Asp—(L)-Phe—OMe	
α-(L)-Asp-(L)-Phe	
β-(L)-Asp—(L)-Phe	
α-(L)-Glu-(L)-Phe—OMe	
α-(L)-Asp-(D)-Phe—OMe	
α-(D)-Asp-(L)-Phe—OMe	
α-(D)-Asp-(D)-Phe—OMe	

*Here and henceforth the degree of sweetness of sucrose was assumed to be unity.

**According to the data in Refs.55 and 83.

IV. RELATION BETWEEN THE STRUCTURE OF A SUBSTANCE AND ITS TASTE IN THE SERIES OF ASPARTAME ANALOGUES

1. Esters of α-aspartyl-containing Dipeptides

Mazur et al.[1] achieved the synthesis of a large series of aspartame analogues (~50 compounds) and reached the first conclusions concerning the structure—taste relation for aspartyl-containing derivatives. The following requirements must be met for a given substance to have a sweet taste: (1) unsubstituted amino- and carboxy-groups must be

present with a specific distance between them; (2) a specific configuration of the asymmetric carbon atom is necessary; (3) an ester group must be present at the C-end of the dipeptide. The authors[1] assumed that, even if only one of these requirements is infringed, the sweet taste disappears (Table 1). Later it was shown that these conditions are not obligatory for the retention of the sweet taste in the series of aspartyl-containing compounds.

When the α-aspartyl residue was replaced by other aminoacids, even one with a similar structure such as glutamic acid, not a single sweet dipeptide was obtained[1]. The explanation is apparently that, for the interaction with the receptor, it is important that that AHB system should be planar, i.e. in the given instance that the amino- and carboxy-groups of the N-terminal aminoacids should form a planar cyclic zwitter-ionic form[44,48] (Fig. 2). This condition can be fulfilled only when the *N*-terminal aminoacid is aspartic or aminomalonic acid. As regards the second part of the aspartame molecule, the latter can undergo various changes to a greater extent. For example, phenylalanine can be replaced by L-tyrosine or L-methionine without loss of the sweet taste.

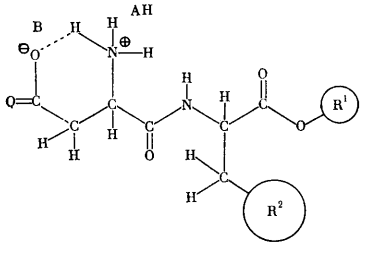
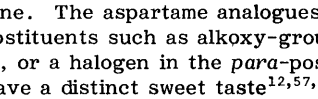


Figure 2. The esters of sweet α-aspartyl-containing dipeptides.

In order to test the requirements imposed on the stereochemistry of the molecule, L–D, D–L-, and D–D-isomers of aspartame were synthesised; they all proved to be bitter. Schlatter[84] showed that the sweet taste does not disappear fully in the DL–DL-, DL–L-, and L–DL-modifications, although the degree of sweetness of these compounds is lower than that of the L–L-isomer.

The data of Mazur et al.[1] demonstrate the influence of the size of the C-terminal ester group on the intensity of the sweet taste, namely the intensity of the sweet taste decreases with increase of the size of the ester group. This shows that variations in the size of the molecule are not unimportant for the taste receptor and confirm the validity of the conclusions of Heijden et al.[43]

Mazur and coworkers[83] extended the list of sweet dipeptides by discovering that these include also the esters of L-α-aspartyl-L-threonine, L-α-aspartyl-*S*-alkyl-L-homocysteine sulphone. The aspartame analogues obtained by introducing substituents such as alkoxy-groups, the hydroxy-group, or a halogen in the *para*-position of the benzene ring have a distinct sweet taste[12,57,83]. The esters of L-α-aspartylphenylglycine are also sweet[92]. It has been observed[10,57,78,93–95] that the partial or complete hydrogenation of the benzene ring in compounds having the general formula

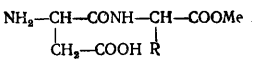


R^1=Alk

R^2=H, OH, OAlk, or Hal

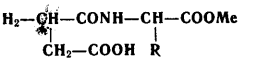
has a favourable effect on the intensity of the sweet taste.

In their study of the contribution of different structural elements to the sweet taste of dipeptide esters, Mazur et al.[95] established that the C-terminal aminoacid can be aliphatic and showed that the intensity of the sweet taste of compounds of the type



varies with the size, form, and hydrophobic properties of the group R [95,96] (Table 2). It was noted that the methyl esters of L–L-dipeptides of this kind do not have a sweet taste as long as the number of carbon atoms in the chain of R does not reach four.

Table 2. The esters of α-aspartyl-containing dipeptides* of the type

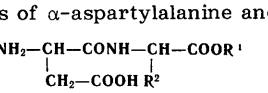


R	RS	Refs.	R	RS	Refs.
Me	0	[95]	n-Pent	50	[95, 96]
iso-Pr	0	[95]	iso-Pent	80	[95, 96]
n-Bu	40	[95, 96]	n-Hex	70	[95, 96]
iso-Bu	0	[95]			

*L–L-configuration.

In the series of α-aspartylalanine and α-aspartyl-*C*-alkylglycine esters, the sweetest dipeptides proved to be those with the L–D-configuration[85,95,97] (Table 3). These compounds are distinguished by the following feature: if the C-terminal ester group R^1 is sufficiently large, as in the case of the n-propyl or isopropyl esters, the group R^2 must be small in order to obtain an intense sweet taste; with increase of its size, the degree of sweetness diminishes[85,95] (Table 3).

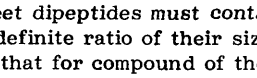
Table 3. The esters of α-aspartylalanine and α-aspartyl-*C*-alkylglycine*:



R^1	R^2	RS	References
Me	Me	25	[95]
Et	Me	80	[95, 97]
n-Pr	Me	170	[85, 95, 97]
iso-Pr	Me	125	[85, 95, 97]
n-Bu	Me	10	[95]
n-Pent	Me	6	[95]
iso-Pr	Et	170	[85, 95, 97]
iso-Pr	iso-Pr	170	[85, 95, 97]
iso-Pr	n-Pr	17	[95]
iso-Pr	s-Bu	4	[95]
iso-Pr	iso-Bu	0	[95]
iso-Pr	n-Bu	0	[95]

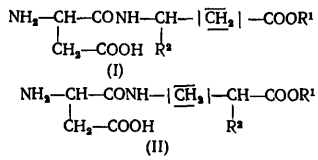
*L–D-configuration.

In general, all sweet dipeptides must contain two hydrophobic groups with definite ratio of their sizes[48] (Fig. 2). It has been shown[98] that for compound of the type



there is a possibility (without loss of sweet taste) of certain combinations of the substitutents R^1 and R^2, which can be aliphatic, alicyclic, or aromatic. It has been noted[99] that the salts of such compounds with certain acids, for example, with malic, citric, etc. acids, are particularly sweet. It has also been recommended that aspartame be used in combination with citric acid[100,101]. The intensification of the taste of dipeptide esters in the presence of citric acid had been observed earlier[10]. Presumably in this case Beidler's hypothesis[23] that the conformation of the membrane of the taste cell varies with the pH of the medium, which can intensify the sweet taste in the presence of the tastant, is valid in this case.

In their search for hydrolytically stable aspartyl derivatives, Miyaoshi et al.[21] synthesised compounds of a new type. In order to rule out the possibility of the formation of substituted dioxopiperazine, they introduced an additional methylene group in various positions along the peptide chain and obtained two types of compounds with an L–D-configuration:

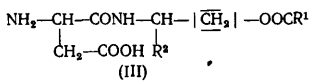


(I)

(II)

Although the length of some of these compounds is similar to that of the n-propyl ester of L-α-aspartyl-D-alanine, the relative sweetness of which amounts to 170 units, the rearrangement of the molecule induced undesirable changes in the structure, which led to a decrease of the level of sweetness to 20 units.

Compounds of type (III), obtained with an increase in chain length, as in compounds (I), with a simultaneous transformation of the terminal carboxy-group, proved more successful:



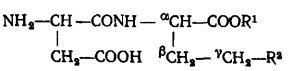
(III)

Eleven *O*-acyl-2-(L-α-aspartylamino)-D-alkanols (III) proved sweeter than sucrose by a factor of 100 and more[21]. The structural requirements which must be met by these compounds as regards the dimensions of the hydrophobic groups R^1 and R^2 are the same as for the dipeptide esters. The configuration of the 2-aminoalkanol esters must correspond to the D-form of the aminoacids; none of the *O*-acyl-2-(α-L-aspartylamino)-L-alkanols proved to be sweet. *N*-(L-α-aspartyl)-*O*-cyclobutyl-D-alaninol is 220 times sweeter than sucrose and, in contrast to aspartame, withstands boiling in aqueous solution at pH 4 and 7 for 1 h without appreciable changes in the taste[21].

The structure–taste relations for α-aspartyl derivatives of hydroxyaminoacids have been investigated[102–104]. The esters of L-α-aspartyl-L-β-(*erythro* and *threo*)-hydroxynorleucine, -*O*-actyl-L-serine, -D-serine, -D-threonine, and -D-allothreonine have been synthesised and their taste compared with that of the corresponding dipeptide esters without a hydroxy-group[103]. It was observed that the introduction of the hydroxy-group has different effects on the taste of the L–L- and L–D-dipeptides: it reduces the sweet taste in the series of L–L-peptide esters and intensifies it in the series of the L–D-isomers. For example, the esters of L-α-aspartyl-D-serine and L-α-aspartyl-D-threonine proved to be sweeter than the corresponding esters of L-α-aspartyl-D-alanine and L-α-aspartyl-D-α-aminobutyric acid. It was also found that the *threo*- and *erythro*-isomers differ in the intensity of the taste[103]. Whereas in the series of the esters of the L–L-dipeptides the sweetest derivatives were the methyl esters[1], among the hydroxy-analogues with the L–D-configuration the n-propyl and isobutyl esters are sweetest[102,103].

The replacement of the methyl group in the side chain in the series of *O*-acyl-*O*-2-α-L-aspartylamino-D-alkanols[21] by the hydroxymethylene group increased the level of sweetness in one case, while in the remaining cases it caused a decrease.

The branching of the side chain by the introduction of even one methyl group in the β- or γ-position resulted in bitter compounds in the series of both hydroxy- and deoxy-derivatives of dipeptide esters[103]:



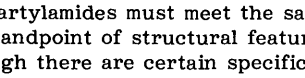
The above data show that the presence of a free or substituted hydroxy-group (depending on its location in the side chain) prevents the hydrophobic binding of the molecule to the receptor only in some cases.

In order to investigate the influence of the nature of the side chain on the intensity of the sweet taste of the esters of aspartyldipeptides, Brussel et al.[104] synthesised a series of structurally similar analogues based on *S*-alkyl-L-cysteine, *O*-alkyl-L-serine, *O*-acyl-L-serine, and asymmetric aminomalonic diesters. The dimensions of the dipeptide side chain were determined on molecular models of these compounds and the characteristics of the shape of the chain were investigated in each specific case. The authors[104] concluded, like Mazur et al.[95], that the intensity of the sweet taste of all the compounds obtained depends on the hydrophobic properties, dimensions, shape, and steric characteristics of the side chain. It has been shown clearly[104] that the degree of branching of the group R^2 in the side chain is not the only important feature, a specific geometrical position of the branching point on the side chain being more significant.

These facts show that the group R^2 of the side chain of the dipeptide ester actually interacts with the hydrophobically binding site of the receptor[43]. This conclusion is valid only in relation to L–L-dipeptides; the hydrophobic binding of the L–D-dipeptides to the receptor is possible only for the C-terminal ester group (because of the different steric dispositions of the substituents at the asymmetric carbon atom of the D-aminoacid), while in *N*-L-α-aspartyl-*O*-acyl-D-aminoalkanols this is possible only for the acyl group. This in fact explains the earlier observation[85,95,97,103] that the degree of sweetness of α-aspartyl derivatives with the L–D-configuration increases with the size of the ester group and with decrease of the bulk of the side chain.

2. α-Aspartylamides

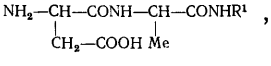
Having synthesised a series of α-aspartylamides, Mazur[105] showed for the first time that the ester group is not an obligatory element in sweet derivatives of aspartic acid; this was later confirmed in relation to aspartylacylaminoalkanols[21], which is not surprising, since the transformations of the terminal carboxy-group in L–L-dipeptides do not involve the main part of the molecule—its glucophore, which is intrinsically involved in the interaction with the receptor. This is why α-aspartylamides must meet the same requirements (from the standpoint of structural features) as dipeptide esters, although there are certain specific differences. To retain the sweet taste, it is necessary that the methyl group be present in the α-position:



Furthermore, when optically active amines are used as the starting materials, steric similarity to L–L-dipeptides must be maintained.

In the series of α-aspartylphenylalkylamides the introduction of a halogen in the *para*-position of the benzene ring as well as hydrogenation of the latter did not intensify the sweet taste[105], as had been observed for dipeptide esters. The aromatic component of aspartylphenylalkylamides can be replaced by an aliphatic chain, as in the case of dipeptide esters. The chain length of the sweetest compounds corresponds to seven carbon atoms; its decrease or increase leads to less sweet or tasteless derivatives. As was to be expected, the form of the chain influences the intensity of the taste. The sweetest α-aspartylamide is α-aspartyl-α,δ-dimethylpentylamide, being 100 times sweeter than sucrose; compounds sweeter than aspartame have not been discovered among them.

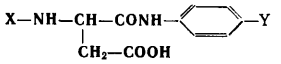
A group of sweet L-α-aspartyl-D-alanylamides of the type



where R^1 = Bu, iso-Bu, s-Bu, or cyclo-Hex, were later synthesised[106]. The level of their sweetness is approximately equal to that of aspartame (they are 100–125 times sweeter than sucrose)[106].

It is of interest to note that the presence of the α-amide linkage in all the known derivatives of aspartic acid is necessary for a sweet taste. If the amide group is alkylated or replaced by an ester group, the taste changes to bitter or disappears respectively; this transformation apparently alters the conformation of the compound so much that a three-point interaction with the receptor becomes impossible[48].

Table 4. N-Acyl-L-α-aspartylanilides:



No.	X	Y	RS	References
1	CF_3CO	H	12	[107—111]
2	CF_3CO	Cl	120	[107, 108, 109, 111]
3	CF_3CO	F	1	[107, 108, 109, 111]
4	CF_3CO	Br	120	[107, 109, 111]
5	CF_3CO	I	*	[107]
6	CF_3CO	CN	3000	[107, 108, 109, 111]
7	CCl_3CO	H	1	[107, 108, 111]
8	CCl_3CO	Cl	**	[108, 111]
9	CCl_3CO	F	**	[108, 111]
10	CCl_3CO	CN	3000	[107, 108, 111]
11	H	CN	12	[107, 111]

*Bitter.

**Sweet product; a quantitative estimate of the RS is not given in the original communications.

3. *N*-Acyl-α-aspartyl Derivatives

The discovery of a sweet taste in certain *N*-acyl derivatives of aspartic acid was quite unexpected[107]. The sweet taste of one of these, namely trifluoroacetyl-L-α-aspartylanalide (No.1 in Table 4), was discovered accidentally[107] (like the taste of aspartame) and could not be explained from the standpoint of the existing ideas concerning the necessity for the presence of a free amino-group in all sweet α-aspartyl derivatives. Not until 1978 was it suggested[46] that the AHB system is mainly required to fix the molecule on the active surface of the receptor. Consequently a single hydrogen bond formed by the carboxy-group may be sufficient to retain the molecule, provided that it is sterically compatible with the receptor surface[46].

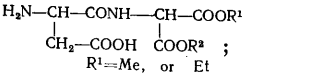
Lapidus and coworkers[107–111] noted the following features characteristic of *N*-acyl-L-α-aspartylanilides (Table 4). The introduction of a single halogen atom (except iodine) or the cyano-group in the *para*-position of the benzene ring of the anilide intensifies the degree of sweetness of the substance. The introduction of a halogen in the *ortho*- and *meta*-positions or the introduction of two halogen atoms does not lead to sweet derivatives. The introduction of substituents such as the methyl, trifluoromethyl, and hydroxy-groups in the *para*-position in the ring also has a negative influence on the taste. Among all the acyl groups tested, only the trifluoroacetyl and trichloroacetyl groups are capable of forming sweet derivatives. These two groups are apparently approximately equivalent as regards their influence on the intensity of the sweet taste (cf. Nos.6 and 10 in Table 4). Comparison of the tastes of individual *N*-acylaspartylanilides (Nos.6 and 10 in Table 4) and their analogues with free amino-groups (No.11 in Table 4) shows that the level of sweetness falls sharply following the removal of acyl groups (from 3000 to 12 units).

As in the series of dipeptide esters, the derivatives of D-aspartic acid lack sweetness entirely[107]. The introduction of the trifluoro- or trichloro-acetyl group into the amino-group of aspartame did not entail either an intensification or a weakening of the taste[107,108,111]. One should note that the synthesis of the *N*-trifluoroacetyl derivatives of aspartame is much simpler than that of aspartame itself; it is achieved in a single stage by the aminolysis of the *N*-trifluoroacetylanhydride of aspartic acid and there is no need to remove protecting groups. Unfortunately, the papers of Lapidus and coworkers[108–111] do not contain information about the methyl ester of *N*-trifluoroacetyl-L-β-aspartyl-L-phenylalanine as a possible side product, although one cannot absolutely rule out its formation when the ring of the corresponding inner anhydride of aspartic acid is opened.

4. The Esters of α-Aspartylaminomalonic Acid

The asymmetric diesters of α-aspartylaminomalonic acid, synthesised by Japanese investigators[112–116], are outstanding among all known sweet compounds by exhibiting the highest level of sweetness. In general aminomalonic acid is unique in the sense that it forms particularly sweet derivatives, occupying either *N*- or C-terminal positions in the dipeptide. For example, the closest analogue of aspartame—the methyl ester of DL-aminomalonyl-L-phenylalanine—surpasses it as regards the intensity of its sweet taste (156–237 units)[112].

Analysis of experimental data[112–116] showed that the ester groups of the aminomalonate must differ significantly from one another in size (Table 5). The sweetest compounds obtained are those in which one of the ester groups is OMe. Specific requirements must be met by the second ester group, since it is responsible for the hydrophobic binding to the receptor. When the R^2 groups in compounds of the type

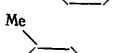
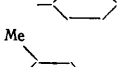


are replaced, the intensity of the sweet taste increases in the following sequence of R^2: aliphatic alkyl < cycloalkyl < cycloalkyl monosubstituted in the 2-position < phenyl. The

introduction of a substituent into any position in the cyclohexyl ring other than the 2-position or an increase of the number of substituents reduce the degree of sweetness[47].

Since all the compounds obtained were racemic mixtures with respect to the aminomalonic residue, it is still not clear which optical isomers in fact exhibit a sweet taste. In order to predict the taste of dipeptide esters, their projection formulae have been compared[117] with those of sweet and non-sweet standards, namely aminoacids, and it was concluded that, in the group of α-aspartylaminomalonic esters, the L–L-isomers should be sweet. This hypothesis has been confirmed by literature data[118].

Table 5. Derivatives of the aminomalonic acid.

L-α-Asp-DL-Ama-OR^1 (OR^2)			
R^1	R^2	RS	References
Me	iso-Pent	500	[115]
Me		300—600	[47, 112, 115]
Me		556—880	[47, 112, 115]
Me	 (*trans*)	5 450—7 300	[47, 112, 116]
Me		22 200—33 200	[47, 112, 113, 114]
Et		128—156	[47]
Et		192—284	[47]
Et	 (*trans*)	524—648	[47]
E		4 200—5 400	[47, 114]

As regards the methyl ester of aminomalonylphenylalanine, some workers[117] believe that the D–L-isomer is sweet, since its conformation agrees best with the receptor model, while others[118] give preference to the L–L-isomer by analogy with aspartame. Studies in this field are being continued and, as soon as the optically pure forms of the above dipeptides are isolated, the discrepancies will be resolved.

---o0o---

It has been shown that the assortment of sweeteners based on aminoacids is fairly wide, their advantages over glucose are in certain aspects incontrovertible, and it may be hoped that in due course universal flavouring agents, meeting most successfully the requirements imposed on synthetic sucrose substituents, will be selected from the above large list and incorporated in the food industry.

REFERENCES

1. R.H.Mazur, J.M.Schlatter, and A.H.Goldkamp, J.Amer.Chem.Soc., **91**, 2684 (1969).
2. Chem.Eng.News, **52**, 5 (1974).
3. R.Franke, Chem.Listy, **72**, 945 (1978).
4. K.M.Beck, in "Low Calorie and Special Dietary Foods" (Edited by B.K.Dwivedi), West Palm Beach, CRC, 1978, p.51.
5. C.Beck, in "Low Calorie and Special Dietary Foods" (Edited by B.K.Dwivedi), West Palm Beach, CRC, 1978, p.59.
6. US P.3 753 739 (1973); Chem.Abs., **80**, 13 818 (1974).
7. BRD P.2 264 394 (1973); Chem.Abs., **80**, 58 607 (1974).
8. E.Davidkova and M.Prudel, Prüm.Potr., **29**, 367 (1978).
9. R.H.Knopp, K.Brandt, and R.A.Arky, J.Toxicol. Environ.Health, **2**, 417 (1976).
10. BRD P.1 948 788 (1970); Chem.Abs., **75**, 19 014 (1971).
11. BRD P.2 227 528 (1973); Chem.Abs., **78**, 102 035 (1973).
12. French P.2 087 843 (1971); Chem.Abs., **77**, 99 898 (1972).
13. G.H.Frey, J.Toxicol.Environ.Health, **2**, 401 (1976).
14. A.E.Harper, in "Sweeteners: Issues Uncertainties", IV, Acad.Forum, Washington, 1975, pp.182, 253.
15. J.W.Olney, in "Sweeteners: Issues Uncertainties", IV, Acad.Forum, Washington, 1975, p.189.
16. Y.Mishiro and H.Kaneko, J.Dent.Res., **56**, 1427 (1977).
17. G.A.Crosby, Critic.Rev.Food Sci.and Nutr., CRC, **7**, 297 (1976).
18. Chem.Eng.News, **56**, 25 (1978).
19. US P.3 971 857 (1976).
20. US P.4 031 259 (1977).
21. M.Miyoshi, K.Nunami, H.Sugano, and T.Fujii, Bull. Chem.Soc.Japan, **51**, 1433 (1978).
22. R.A.Pevzner, Symposium, "Sensornye Sistemy" (Sensor Systems) (Edited by G.V.Gershuni), Izd. Nauka, Leningrad, 1978, p.115.
23. K.Kurihara and L.M.Beidler, Nature, **222**, 1176 (1969). (1969).
24. T.N.Bratus', M.V.Kozlova, and V.A.Lebedeva, Symposium, "Sensornye Sistemy" (Sensor Systems) (Edited by G.V.Gershuni), Izd.Nauka, Leningrad, 1978, p.138.
25. F.R.Dastoli and S.Price, Science, **154**, 905 (1966).
26. F.R.Dastoli, D.V.Lopiekes, and S.Price, Biochem., **7**, 1169 (1968).
27. F.R.Dastoli, D.V.Lopiekes, and A.Doig, Nature, **218**, 884 (1968).
28. S.Price, J.Agr.Food Chem., **17**, 709 (1969).
29. Ya.A.Vinnikov, "Tsitologicheskie i Molekulyarnye Osnovy Retseptsii" (Cytological and Molecular Principles of Reception), Izd.Nauka, Leningrad, 1971, p.122.
30. L.M.Beidler, J.Gen.Physiol., **38**, 133 (1954).
31. L.M.Beidler, J.Food Sci., **31**, 271 (1966).
32. L.M.Beidler, Adv.Chem., Ser.56, 1966, p.1.
33. R.H.Cagan, Biochim.Biophys.Acta, **252**, 199 (1971).
34. R.S.Shallenberger and T.E.Acree, Nature, **216**, 480 (1967).
35. J.Solms, J.Agr.Food Chem., **17**, 686 (1969).
36. R.S.Shallenberger, T.F.Acree, and C.Y.Lee, Nature, **221**, 555 (1969).
37. H.-D.Belitz and H.Wieser, Z.Lebensm.Unters.-Forsch., **160**, 251 (1976).
38. H.Wieser, H.Jugel, and H.-D.Belitz, Lebensm.Unters.-Forsch., **164**, 277 (1977).
39. F.P.A.Lehmann, Life Sci., **22**, 1631 (1978).

40. L.B.Kier, J.Pharm.Sci., **61**, 1384 (1972).
41. H.D.Höltje and L.B.Kier, J.Pharm.Sci., **63**, 1722 (1974).
42. E.W.Deutsch and C.Hansch, Nature, **211**, 75 (1966).
43. A.Heijden, L.B.P.Brussel, and H.G.Peer, Food Chem., **3**, 207 (1978).
44. F.Lelj, T.Tancredi, P.A.Temussi, and C.Toniolo, J.Amer.Chem.Soc., **98**, 6669 (1976).
45. F.Lelj, T.Tancredi, P.A.Temussi, and C.Toniolo, in "Peptides 1976, Proceedings of the XIVth European Peptide Symposium" (Edited by A.Loffet), Brussels, 1976, p.585.
46. P.Temussi, F.Lelj, and T.Tancredi, J.Medicin.Chem., 21, 1154 (1978).
47. M.Fujino, M.Wakimasu, M.Mano, K.Tanaka, N.Nakajima, and H.Aoki, Chem.Pharm.Bull.(Tokyo), **24**, 2112 (1976).
48. M.Goodman and C.Gilon, in "Peptides 1974" (Edited by Y.Wolman), Halsted Press, New York, 1975, p.271.
49. G.H.Dodd, in "Structure-Activity Relationships in Chemoreception, Proc.Symp.1975" (Edited by G.Benz), London, 1976, p.2.
50. L.Kier, in "Structure-Activity Relationships in Chemoreception, Proc.Symp.1975" (Edited by G.Benz), London, 1976, p.101.
51. L.H.Hall and L.B.Kier, J.Pharm.Sci., **66**, 642 (1977).
52. C.Y.Lee, S.E.Mammai, and G.G.Birch, J.Food Sci., **40**, 390 (1975).
53. F.Pautet and C.Nofre, Z.Lebensm.Unters.-Forsch., **166**, 167 (1978).
54. I.B.Ostretsova, Biokhimiya, **43**, 1037 (1978).
55. Swiss P.508 590 (1971).
56. Japanese Appl.76 836 (1973); Chem.Abs., **80**, 48 405 (1974).
57. Dutch P.7 007 176 (1971); Chem.Abs., **76**, 86 150 (1972).
58. B.P.1 339 101 (1973); Chem.Abs., **80**, 96 372 (1974).
59. BRD P.2 256 055 (1973); Chem.Abs., **79**, 42 847 (1973).
60. BRD P.2 452 285 (1975); Chem.Abs., **83**, 79 615 (1975).
61. Y.Ariyoshi, T.Yamatani, N.Uchiyama, Y.Adachi, and N.Sato, Bull.Chem.Soc.Japan, **46**, 1893 (1973).
62. Y.Ariyoshi, T.Yamatani, and Y.Adachi, Bull.Chem. Soc.Japan, **46**, 2611 (1973).
63. Japanese Appl.18 249 (1973); Chem.Abs., **78**, 160 119 (1973).
64. US P.3 901 871 (1975).
65. BRD P.2 233 535 (1973); Chem.Abs., **78**, 98 023 (1973).
66. B.P.1 481 186 (1976).
67. US Appl.485 972 (1976); Chem.Abs., **85**, 6055 (1976).
68. US P.3 808 190 (1974).
69. Japanese Appl.67 243 (1973); Chem.Abs., **80**, 27 474 (1974).
70. BRD P.2 326 897 (1972); Chem.Abs., **80**, 60 216 (1974).
71. US P.3 833 554 (1974).
72. BRD P.2 152 111 (1970); Chem.Abs., **77**, 20 029 (1972).
73. Japanese Appl.13 737 (1976); Chem.Abs., **85**, 6056 (1976).
74. Y.Ariyoshi and N.Sato, Bull.Chem.Soc.Japan, **45**, 942 (1972).
75. Y.Ariyoshi and N.Sato, Bull.Chem.Soc.Japan, **45**, 2015 (1972).
76. Japanese P.25 190 (1973); Chem.Abs., **80**, 3798 (1974).
77. I.Furda, P.D.Malizia, M.G.Kolov, and P.I.Vernieri, J.Agr.Food.Chem., **23**, 340 (1975).
78. B.P.1 298 700 (1972); Chem.Abs., **78**, 111 764 (1973).
79. Japanese P.00 812 (1973); Chem.Abs., **78**, 148 238 (1973).
80. Japanese Appl.96 557 (1973); Chem.Abs., **80**, 96 371 (1974).
81. Japanese P.42 491 (1974); Chem.Abs., **83**, 10 848 (1975).
82. H.Pietsch, Tetrahedron Letters, 4053 (1976).
83. US P.3 475 403 (1969).
84. US P.3 492 131 (1970).
85. French P.2 114 657 (1972); Chem.Abs., **78**, 84 821 (1973).
86. E.Schroeder and K.Luebke, "Peptides" (Translated into Russian), Izd.Mir, Moscow, 1967, Vol.1, p.116.
87. L.A.Pavlova, S.M.Andreev, Yu.A.Davidovich, and S.M.Rogozhin, USSR P.713 863 (1978); Byul.Izobret., No.5, 75 (1980).
88. L.A.Pavlova, S.M.Andreev, Yu.A.Davidovich, and S.M.Rogozhin, USSR P.713 862 (1978); Byul.Izobret., No.5, 75 (1980).
89. BRD Appl.2 608 174 (1977).
90. US P.4 021 418 (1976); Chem.Abs., **85**, 124 370 (1976).
91. Food Eng., **50**, 40 (1978).
92. US P. 3 972 860 (1976).
93. Canadian P.948 185 (1974); Chem.Abs., **82**, 86 641 (1975).
94. BRD P.1 936 159 (1970); Chem.Abs., **72**, 101 098 (1970).
95. R.H.Mazur, J.A.Reuter, K.A.Swiatek, and J.M.Schlatter, J.Medicin.Chem., **16**, 1284 (1973).
96. US P.3 799 918 (1974).
97. US P.3 853 835 (1973); Chem.Abs., **81**, 58 606 (1974).
98. BRD P.2 054 545 (1971); Chem.Abs., **75**, 77281 (1971).
99. BRD P.2 315 646 (1973); Chem.Abs., **81**, 58 606 (1974).
100. US P.3 956 507 (1976); Chem.Abs., **85**, 45 178 (1976).
101. US P.4 004 039 (1977); Chem.Abs., **86**, 119 534 (1977).
102. US P.3 798 204 (1974).
103. Y.Ariyoshi, N.Yasuda, and T.Yamatani, Bull.Chem. Soc.Japan, **47**, 326 (1974).
104. L.B.P.Brussel, H.G.Peer, and A.Heijden, Z.Lebensm. Unters.-Forsch., **159**, 337 (1975).
105. R.H.Mazur, A.H.Goldkamp, P.A.James, and J.M.Schlatter, J.Medicin.Chem., **13**, 1217 (1970).
106. M.Sukehiro, H.Minematsu, and K.Noda, Seikatsu Kagaku, **11**, 9 (1977).
107. M.Lapidus and M.Sweeney, J.Medicin.Chem., **16**, 163 (1973).
108. US P.3 725 453 (1973); Chem.Abs., **79**, 53 780 (1973).
109. US P.3 769 333 (1973); Chem.Abs., **80**, 71 097 (1974).
110. US P.3 814 747 (1974).
111. US P.3 818 077 (1974).
112. M.Fujino, M.Wakimasu, K.Tanaka, H.Aoki, and N.Nakajima, Naturwiss., **60**, 351 (1973).
113. BRD P.2 335 010 (1974); Chem.Abs., **80**, 121 331 (1974).
114. US P.3 907 766 (1975).
115. US P.3 801 563 (1974).
116. US P.3 959 245 (1976).
117. Y.Ariyoshi, Agr.Biol.Chem., **40**, 983 (1976).
118. M.Fujino, M.Wakimasu, K.Tanaka, H.Aoki, and N.Nakajima, "Proceedings of the XIth Symposium on Peptide Chemistry" (Edited by H.Kotake), Tokyo, 1973, p.103.

Institute of Organoelemental
Compounds, USSR Academy
of Sciences, Moscow