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PATENT SPECIFICATION

NO DRAWINGS

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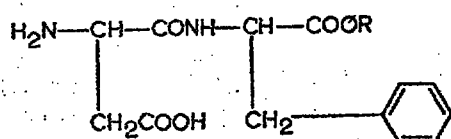
COMPLETE SPECIFICATION

Sweetening Agents

We, G. D. SEARLE & Co., a corporation organised and existing under the laws of the State of Delaware, United States of America, of Post Office Box 5110, Chicago, Illinois 60680, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to novel compositions which are especially useful in view of their sweetening properties and to novel methods for the use of those compositions as sweetening agents.

The substances which exhibit this surprising sweetening property are dipeptide derivatives of the following structural formula



(I)

wherein R is an alkyl radical containing from 1 to 7 carbon atoms.

The alkyl radicals denoted by the R term are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl and the branched-chain groups isomeric therewith.

The surprisingly potent sweet taste of these dipeptide derivatives is completely unexpected and could not have been predicted from a consideration of their chemical structure. That property apparently is related to

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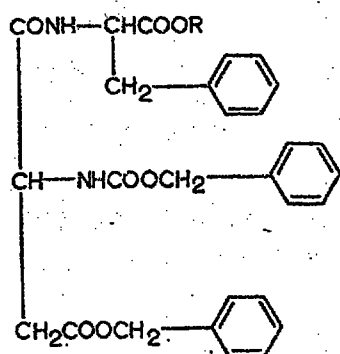
the polarity of the molecule as indicated by the fact that the compound wherein R is a hydrogen atom, i.e. the corresponding free carboxylic acid, is completely lacking in sweetness.

The sweetening property of the dipeptide substances is dependant also upon the stereochemistry of the individual amino acids, i.e. aspartic acid and phenylalanine, from which the dipeptides are derived. Each of the amino acids can exist in either the D or L form, but it has been determined that the L - aspartyl - L - phenylalanine esters are sweet while the corresponding D-D, D-L, and L-D isomers are not. Combinations of isomers which contain the L-L dipeptide, i.e. DL - aspartyl - L - phenylalanine, L - aspartyl - DL - phenylalanine and DL - aspartyl - DL phenylalanine esters, are also sweet.

The dipeptide esters used in the present invention are conveniently manufactured by methods suitable for the coupling of amino acids. An especially preferred starting material is the L - aspartic acid derivative wherein the amino function is protected by a benzyl - oxycarbonyl group and the β - carboxy function by a benzyl ester group, and the α - carboxy group is converted to a p - nitrophenyl ester function. The preparation of that substance, i.e. N - benzyl-oxycarbonyl - L - aspartic acid α - p - nitrophenyl, β - benzyl diester, is described by S. Guttmann, *Helv. Chim. Acta*, 44, 721 (1961). When that substance is allowed to react with a phenylalanine ester, displacement of the more reactive p - nitrophenyl ester group occurs to afford the protected dipeptide of the following formula

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(II)

wherein R is a lower alkyl radical as defined above. Removal of the N - benzyloxycarbonyl and O - benzyl protecting groups is conveniently effected by hydrogenolysis at atmospheric pressure and room temperature, utilizing palladium as the catalyst. Those processes are specifically illustrated by the reaction of N - benzyl - oxycarbonyl - L - aspartic acid α - p - nitrophenyl, β - benzyl diester with L - phenylalanine methyl ester to afford β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester and hydrogenolysis of that intermediate in aqueous acetic acid with palladium metal catalyst to produce L - aspartyl - L - phenylalanine methyl ester.

The sweetening agents used in the present invention exhibit a remarkably greater potency than does sucrose. The sweetness of an aqueous solution of L - aspartyl - L - phenylalanine methyl ester, for example, can be detected at a concentration between 0.5—1% of that of sucrose. In other words, that dipeptide derivative in aqueous solution is about 100—200 times as sweet as sucrose. The corresponding ethyl ester is 25—50 times as potent as sucrose. Upon addition of L - aspartyl - L - phenylalanine methyl ester to edible food products, the resultant materials have exhibited a sweetness potency of the order of 200—300 times that of identical materials sweetened with sucrose. It is apparent that the sweetening agents of the present invention will be particularly useful to diabetics and persons for whom it is desirable to restrict the intake of products sweetened with high caloric sweeteners such as sucrose.

These dipeptide esters of formula (I) provide further advantages in that they do not result in the unpleasant after-taste (e.g. bitter, metallic-saline taste) characteristic of synthetic sweeteners such as saccharin and cyclamate, but do contribute a greater flavour fullness than that of the aforementioned synthetic sweeteners. In consequence of their derivation from natural sources, i.e. natural occurring amino acids, the sweeteners used

in the present invention are devoid of toxic properties.

The dipeptide sweetening agents of the present invention can be usefully employed to impart sweetness to edible materials. The term "edible materials" as used herein signifies all non-toxic substances consumable by humans and other animals in solid or liquid form. Illustrative of such substances are foods, including food-stuffs, prepared food items, chewing gum, and beverages; food additives, including flavouring and colouring agents as well as flavour enhancers; and drugs. The compounds of formula (I) are water-soluble, stable substances which can be prepared in a variety of forms suitable for the utilisation as sweetening agents. Typical forms which can be employed are tablets, powders suspensions, solutions and syrups as well as other commonly employed forms particularly suited for combination with edible materials. These forms can consist of the compounds of formula (I) apart or in association with non-toxic sweetening agent carriers, i.e. non-toxic substances commonly employed in association with sweetening agents. Such suitable carriers include liquids such as water, ethanol, glycerol, corn oil, sesame oil, propylene glycol, corn syrup, and liquid paraffin, and solids such as lactose, sorbitol, citric acid, cellulose, starch, salt, and glucose. Obviously incompatible for use with the sweetening agents used in the present invention would be toxic carriers such as methanol and dimethyl sulphoxide.

Examples of specific edible materials which can be sweetened by the addition of a compound of formula (I) apart or in combination with a non-toxic sweetening agent carrier are: fruits; vegetables; juices; meat products such as ham, bacon, and sausage; egg products; fruit concentrates; salad dressings; milk products such as ice cream and sherbert; icings; syrups; corn; wheat and rice products such as bread, cereals, and cake mixes; fish; cheese and cheese products; nut products; beverages such as coffee, tea, carbonated soft drinks, beers and wines; and confections such as candy. Illustrations of the preparation of four such sweetened products are given below.

Coffee

To regular grind hot brewed coffee was added a sample of L - aspartyl - L - phenylalanine methyl ester until the content of the dipeptide in the solution reached 0.015%. Upon comparison with similarly prepared solutions of coffee sweetened with sucrose it was found that to achieve the same degree of sweetness a 4% solution of sucrose was required. Thus in black coffee the dipeptide of formula (I) exhibited a sweetness potency of 270 times that of sucrose.

Fresh Grapefruit

A supply of freshly cut grapefruit was prepared. The supply was divided into equal parts and L - aspartyl - L - phenylalanine methyl ester and sucrose were added. It was found that 0.03% of the dipeptide was required to achieve the sweetness produced by 7% of sucrose. Thus, in fresh grapefruit, the dipeptide exhibited a potency of 230 times the sweetness of sucrose.

Powder Beverage Concentrate

The powder was prepared by mixing 0.05 parts and L - aspartyl - L - phenylalanine strawberry flavouring 0.033 parts of L - aspartyl - L - phenylalanine methyl ester, and 0.609 part of lactose. The powder was added to 99.268 parts of spring water, dissolved and the beverage evaluated at room temperature. This sample was compared to a similar sample, prepared as indicated above except that 9 parts sucrose and 0.87 part dextrose were substituted for the dipeptide ingredient. Upon tasting it was determined that the two samples achieved the same degree of sweetness and hence it was found that in powder concentrates of this type, the compound of formula (I) exhibited 270 times the sweetness potency of sucrose.

Gelatine Dessert

A sample was prepared from 2.07 parts of plain gelatine, 0.34 parts of imitation raspberry flavouring, 0.34 part of citric acid, 14.41 parts of lactose and 0.05 part of L - aspartyl - L - phenylalanine methyl ester.

The ingredients were combined and dissolved in 82.79 parts of boiling spring water. Thereafter the gelled solution was poured into dishes and chilled to set. Similarly prepared was a gelatin sample containing sucrose in place of the dipeptide of formula (I) and the lactose. Upon taste comparison of the cold gelatin samples it was found that a sucrose sample containing 14.5% of sucrose was required in order to achieve the sweetness of a dipeptide sample containing 0.05% of the above named dipeptide. Thus, in gelatin desserts, the compound of formula (I) i.e. L - aspartyl - L - phenylalanine methyl ester, exhibited 290 times the sweetness potency of sucrose.

The following examples illustrate the preparation of the dipeptide compounds of formula (I). In these examples temperatures are given in degrees centigrade ($^{\circ}\text{C}$) and quantities of materials are expressed in parts by weight except where otherwise noted.

EXAMPLE 1

A solution of 88.5 parts of L - phenyl - alanine methyl ester hydrochloride in 100 parts of water was neutralised by the addition of dilute aqueous potassium bicarbonate, then was extracted with approximately

900 parts of ethyl acetate. The resulting organic solution was washed with water and dried over anhydrous magnesium sulphate. To that solution was then added 200 parts of N - benzyloxycarbonyl - L - aspartic acid α - p - nitrophenyl, β - benzyl diester, and that reaction mixture was kept at room temperature for about 24 hours, then at approximately 65° for about 24 hours. The reaction mixture was cooled to room temperature, diluted with approximately 390 parts of cyclohexane, then cooled to approximately -18° in order to complete crystallisation. The resulting crystalline product was isolated by filtration and dried to afford β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester, melting at about 118.5 — 119.5° .

EXAMPLE 2

A solution of 6.15 parts of L - phenyl - alanine n - propyl ester hydrochloride in 20 parts of water was neutralised by the addition of dilute aqueous potassium bicarbonate, then was extracted with approximately 45 parts of ethyl acetate. That organic solution was washed with water and dried over anhydrous magnesium sulphate. A quantity of N - benzyloxycarbonyl L - aspartic acid α - p - nitrophenyl, β - benzyl diester amounting to 10.05 parts was added, and the resulting reaction mixture was kept at room temperature for about 5 days. Washing of that mixture successively with dilute hydrochloric acid, water, dilute aqueous potassium carbonate and water afforded an organic solution which was dried over anhydrous magnesium sulphate, then evaporated to dryness under reduced pressure. The resulting solid residue was purified by recrystallisation from cyclohexane - isopropyl acetate to yield β - benzyloxycarbonyl - L - aspartyl - L - phenylalanine n - propyl ester, which melted at about 90 — 91° .

EXAMPLE 3

When 8.2 parts of L - phenylalanine ethyl ester hydrochloride and 17.1 parts of N - benzyloxycarbonyl - L - aspartic acid α - p - nitrophenyl, β - benzyl diester were substituted in the procedure of Example 2, there was obtained a solid crude product, which was purified by recrystallisation from isopropyl alcohol to afford β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine ethyl ester, melting at about 89 — 94° .

EXAMPLE 4

When the procedure of Example 2 was repeated with the substitution of 6.14 parts of L - phenylalanine isopropyl ester hydrochloride, there was obtained a solid crude product which was purified by recrystallisation from ethyl acetate - cyclohexane to yield

β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine isopropyl ester, melting at about 95—101°.

EXAMPLE 5

5 A solution containing 4.78 parts of N - benzyloxycarbonyl L - aspartic acid α - p - nitrophenyl, β - benzyl diester and 2.63 parts of L - phenylalanine tertiary butyl ester in 18 parts of ethyl acetate was stored at room temperature for about 6 days, then was washed successively with dilute hydrochloric acid, water, dilute aqueous potassium carbonate and water. Drying over anhydrous magnesium sulphate followed by evaporation of the solvent under reduced pressure afforded the crude product, which was purified by recrystallisation from hexane - carbon tetra- chloride to yield β - benzyl N - benzyl - oxycarbonyl - L - aspartyl - L - phenyl - alanine tertiary butyl ester, which melted at about 69—70.5°.

EXAMPLE 6

To a solution of 180 parts of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester in 3000 parts by volume of 75% acetic acid was added 18 parts of palladium black metal catalyst, and the resulting mixture was shaken with hydrogen at atmospheric pressure and room temperature for about 12 hours. The catalyst was removed by filtration, and the solvent was distilled under reduced pressure to afford a solid residue, which was purified by recrystallisation from aqueous ethanol to yield L - aspartyl - L - phenylalanine methyl ester. It displays a double melting point at about 190° and 245—247°.

EXAMPLE 7

40 A mixture containing 9.6 parts of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine ethyl ester, one part of palladium black metal catalyst and 1,000 parts by volume of 75% acetic acid was shaken with hydrogen at atmospheric pressure and room temperature for about 2 hours. The catalyst was removed by filtration, and the resulting filtrate was distilled to dryness under reduced pressure. Recrystallisation of the solid residue from water afforded L - aspartyl L - phenylalanine ethyl ester, which exhibited a double melting point at about 184° and 245—246°.

EXAMPLE 8

55 A mixture containing 5.3 parts of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine n - propyl ester and 0.5 part of palladium black metal catalyst with 100 parts by volume of 75% acetic acid was shaken with hydrogen at atmospheric pressure and room temperature for about 1½ hours at the end of which time the catalyst was re-

moved by filtration. The resulting filtrate was stripped of solvent by distillation under reduced pressure, and the resulting residue was purified by recrystallisation from aqueous methanol to yield L - aspartyl - L - phenylalanine n - propyl ester. This compound exhibits a double melting point at about 155—160° and 242—245°.

EXAMPLE 9

When 7 parts of β - benzyl N - benzyloxycarbonyl - L - aspartyl L - phenylalanine isopropyl ester was hydrogenated according to the procedure of Example 8, there was produced L - aspartyl - L - phenylalanine isopropyl ester. This compound exhibited a double melting point at about 180—181° and 232—235°.

EXAMPLE 10

To a solution of 2.2 parts of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine tertiary butyl ester in 75 parts by volume of 75% acetic acid was added 0.2 part of palladium black metal catalyst, and that reaction mixture was shaken with hydrogen at atmospheric pressure and room temperature for about one hour. Following that reaction period, the catalyst was removed by filtration, and the solvent was evaporated under reduced pressure to afford a solid residue. That residue was dissolved in hot water, then is lyophilised to afford L - aspartyl - L - phenylalanine tertiary butyl ester, melting at about 143—148°.

EXAMPLE 11

When an equivalent quantity of DL - phenylalanine methyl ester was substituted in the procedure of Example 1, there was produced a mixture of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester and β - benzyl N - benzyloxycarbonyl - L - aspartyl - D - phenylalanine methyl ester.

EXAMPLE 12

The substitution of an equivalent quantity of N - benzyloxycarbonyl - DL - aspartic acid α - p - nitrophenyl, β - benzyl diester in the procedure of Example 1 afforded a mixture of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester and β - benzyl N - benzyloxycarbonyl - D - aspartyl - L - phenylalanine methyl ester.

EXAMPLE 13

The substitution of equivalent quantities of N - benzyloxycarbonyl - DL - aspartic acid α - p - nitrophenyl, β - benzyl diester and DL - phenylalanine methyl ester in the procedure of Example 1 resulted in a mixture of β - benzyl N - benzyloxycarbonyl - DL - aspartyl - DL - phenylalanine methyl

ester and β - benzyl N - benzyloxycarbonyl - DL - aspartyl - LD - phenyl - alanine methyl ester.

EXAMPLE 14

- 5 By substituting an equivalent quantity of the mixture of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester and β - benzyl N - benzyloxycarbonyl - L - aspartyl D - phenylalanine methyl ester and otherwise proceeding according to the processes of Example 6, there was produced a mixture of L - aspartyl - L - phenyl - alanine methyl ester and L - aspartyl - D - phenylalanine methyl ester.

EXAMPLE 15

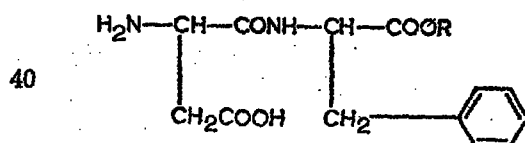
- 15 When an equivalent quantity of the mixture of β - benzyl N - benzyloxycarbonyl - L - aspartyl - L - phenylalanine methyl ester and β - benzyl N - benzyloxycarbonyl - D - aspartyl - L - phenylalanine methyl ester was substituted in the procedure of Example 6, there was produced a mixture of L - aspartyl - L - phenylalanine methyl ester and D - aspartyl - L - phenylalanine methyl ester.

EXAMPLE 16

- 20 The substitution of an equivalent quantity of the mixture of β - benzyl N - benzyloxycarbonyl - DL - aspartyl - DL - phenyl - alanine methyl ester and β - benzyl N - benzyloxycarbonyl - DL - aspartyl - LD - phenylalanine methyl ester in the procedure of Example 6 resulted in a mixture of DL - aspartyl - DL - phenylalanine methyl ester and DL - aspartyl - LD - phenylalanine methyl ester.

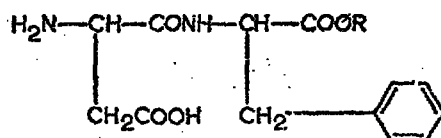
WHAT WE CLAIM IS:—

1. A sweetening composition comprising a compound of the general formula



wherein R is an alkyl radical containing from 1 to 7 carbon atoms and the stereochemical configuration is DL-DL, L-L, DL-L, or L-DL and an edible material.

- 45 2. A sweetening composition comprising a compound of the general formula



- 50 wherein R is an alkyl radical containing from 1 to 7 carbon atoms and the stereochemical configuration is DL-DL, L-L, DL-L or L-DL

and a non-toxic sweetening agent carrier therefor with an edible material.

3. A composition as claimed in any one of the preceding claims wherein the compound is L - aspartyl - L - phenylalanine methyl ester.

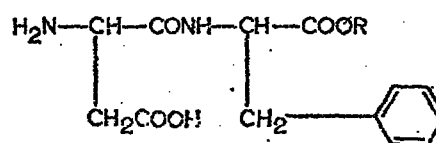
4. A composition as claimed in any one of the preceding claims 1 or 2 wherein the compound is L - aspartyl - L - phenyl - alanine ethyl ester.

5. A composition as claimed in any one of the preceding claims 1 or 2 wherein the compound is L - aspartyl - L - phenyl - alanine n - propyl ester.

6. A composition as claimed in any one of the preceding claims 1 or 2 wherein the compound is L - aspartyl - L - phenyl - alanine isopropyl ester.

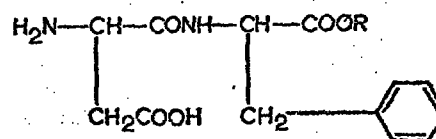
7. A composition as claimed in any one of the preceding claims 1 or 2, wherein the compound is L - aspartyl - L - phenyl - alanine tertiary butyl ester.

8. A method for sweetening edible materials which comprises adding thereto a compound of the general formula



wherein R is an alkyl radical containing from 1 to 7 carbon atoms and the stereochemical configuration is DL-DL, L-L, DL-L or L-DL.

9. A method for sweetening edible materials which comprises adding thereto a composition comprising a compound of the general formula



wherein R is an alkyl radical containing from 1 to 7 carbon atoms and the stereochemical configuration is DL-DL, L-L, DL-L or L-DL and a non-toxic sweetening agent carrier therefor.

10. A method as claimed in claim 8 or 9 wherein the compound is L - aspartyl - L - phenylalanine methyl ester.

11. A method as claimed in claim 8 or 9 wherein the compound is L - aspartyl - L - phenylalanine ethyl ester.

12. A method as claimed in claim 8 or 9 wherein the compound is L - aspartyl - L - phenylalanine n - propyl ester.

13. A method as claimed in claim 8 or 9 wherein the compound is L - aspartyl - L - phenylalanine isopropyl ester.

(22)

14. A method as claimed in claim 8 or 9 wherein the compound is L - aspartyl - L - phenylalanine tertiary butyl ester.

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ERRATA

SPECIFICATION No. 1,152,977

Page 3, line 13, *delete* "and L - aspartyl - L - phenylalanine" *substitute* "of citric acid, 0.04 part of imitation"

Page 3, line 103, *after* " β -" *insert* "benzyl N-"

THE PATENT OFFICE

23rd June 1969