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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

A process for producing 5'-Nucleotides and/or Nucleosides

5 We, TAKEDA PHARMACEUTICAL INDUSTRIES, LTD., of 27, 2-chome, Doshomachi, Higashi-ku, Osaka, Japan, a Body Corporate organised under the Laws of Japan, 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:—

10 This invention relates to the production of 5'-nucleotides.

15 Nucleic acids, which are widely distributed in living bodies as non-protein components in nucleoproteins and which are closely concerned with living phenomena, are essential components of living bodies and are constructed of various nucleotides. Of these nucleotides, 5'-nucleotides are important compounds as useful reagents for biological researches and as a seasoning for food.

20 In spite of the wide distribution of 5'-nucleotides in natural sources as components of ribonucleic acids, 5'-nucleotides have been regarded as compounds hardly obtainable in a pure state, because no suitable method has yet been found for hydrolysing ribonucleic acids into 5'-nucleotides; accordingly the use of 5'-nucleotides has been extremely limited up to the present.

30 Both chemical and biochemical processes for hydrolysing ribonucleic acids are known. The chemical process, however, does not give 5'-nucleotides, but rather 2'- and/or 3'-nucleotides. One of the biochemical processes which is known is a process in which ribonucleic acids or products obtained by hydrolysing ribonucleic acid with pancreatic ribonuclease are further hydrolysed with phosphodiesterase from snake venoms or from the mucous mem-

brane of bovine small intestines. This method, however, has such deficiencies as giving only a low yield of 5'-nucleotides and as the difficulty of obtaining the enzymes to be used. This method, therefore, is not readily applicable to the industrial production of 5'-nucleotides.

40 The present invention provides a process for the production of 5'-nucleotides, which comprises contacting one or more ribonucleic acids and/or oligoribonucleotides at a pH between 4 and 10 with living cells of, or an enzyme system produced by, a microorganism belonging to the genus *Streptomyces* in the absence of phosphomonoesterase or in the presence of a phosphomonoesterase inhibitor, (which inhibitor may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced. The product obtained by this process may be added as a seasoning to food.

50 The invention also provides a process for the production of 5'-nucleotides, which comprises contacting one or more ribonucleic acids and/or oligoribonucleotides at a pH between 4 and 10 with living cells of, or an enzyme system produced by, a microorganism belonging to the class *Fungi imperfecti* in the absence of phosphomonoesterase or in the presence of a phosphomonoesterase inhibitor, (which inhibitor may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced.

60 Deaminase may be present during the hydrolysis although this may lead to contamination of the product.

70 Ribonucleic acids which are used as starting materials for the process of this invention may be obtained from such natural sources as yeast

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and animal tissues, and, oligoribonucleotides obtained by the partial hydrolysis of ribonucleic acids may also be employed as the starting material. Furthermore, unrefined ribonucleic acid- or oligoribonucleotide-containing substances such as aqueous yeast extracts, may also be used as the starting material. The products of this invention are 5'-mononucleotides and comprise adenylic acid, cytidylic acid, uridylic acid, guanylic acid and inosinic acid.

The products obtained are mixtures and, as such, have various uses as described above but, if necessary, each component may be separated, using methods which are known *per se*. The 5'-nucleotides obtained by the process of the invention, together with an inert carrier, may be used as seasonings for food.

For the purpose of producing 5'-nucleotides according to this invention, various kinds of microorganisms, or enzymes produced thereby, may be utilised. The microorganisms which may be used belong to the genus *Streptomyces* or the class *Fungi imperfecti*. Examples of the class *Fungi imperfecti* are *microorganisms* belonging to the genera *Fusarium*, *Verticillium*, *Gliomastix* and *Helminthosporium*. Preferred examples of the microorganisms which may be used are:

Fusarium roseum Link

Fusarium solani (Mart.) Appel et Wollenweber

Verticillium niveostratosum Lindau

Gliomastix convoluta (Harz.) Mason var. *felina* (Marchal) Mason

Helminthosporium sigmoideum var. *irregularare* Gralley et Tullis

Streptomyces griseus (Krainsky emend. Waksman et al) Waksman et al.

Streptomyces coelicolor (Müller) Waksman et Henrici

Streptomyces flavus (Krainsky) Waksman et Henrici

Streptomyces lavendulae (Waksman et Curtis) Waksman et Henrici

Streptomyces ruber (Krainsky) Waksman et Henrici

Streptomyces viridochromogenes (Krainsky) Waksman et Henrici

Streptomyces purpureus Lindenbeim

Streptomyces albogriseolus Benedict et al.

Streptomyces olivochromogenus (Bergey et al.) Waksman et Henrici

Streptomyces aureus (Waksman et Curtis) Waksman et Henrici

Streptomyces gougeroti (Duche) Waksman et Henrici

Streptomyces griseoflavus (Krainsky) Waksman et Henrici

Strains of these microorganisms are all preserved at the Institute for Fermentation, Osaka (I.F.O.) and are all available therefrom, and they also are easily obtainable from such culture collections in various countries as the American Type Culture Collection (A.T.C.C.) Washington, D.C., United States of America,

Northern Utilization Research Branch of United States Department of Agriculture, Peoria, Illinois, United States of America, and Centraal bureau voor Schimmelcultures, Baarn, Holland.

In the process of the present invention, a microorganism as exemplified above, or phosphodiesterase produced thereby, is subjected to a reaction with ribonucleic acids or with oligoribonucleotides. When the microorganisms themselves are utilised, the hydrolysis of ribonucleic acid or oligoribonucleotides is caused by phosphodiesterase produced by the microorganisms. Therefore, the method of this invention may be regarded as a method for hydrolysing ribonucleic acids or oligoribonucleotides into 5'-nucleotides by means of phosphodiesterase of such microorganisms.

These phosphodiesterases may be used in the state of a component in living mycelia or a culture filtrate of the microorganisms, a suspension of their microbial cells or as extracted enzymes. The extracted enzymes may be used in a crude or pure state. To bring the phosphodiesterases in the living cells of the microorganism into contact with ribonucleic acids or oligoribonucleotides, it is desirable to incubate the microorganisms on a nutrient medium containing the starting material or to add the starting material into the culture of the microorganisms subsequently. Incubation in the above cases may be conducted in a liquid or solid medium, and the medium may be stirred with aeration or may not be stirred at all. The most convenient and advantageous process, however, is the so-called submerged culture with aeration and agitation.

When the processes of the present invention are effected by a culture method, the most suitable medium is selected in accordance with the kind and nature of the microorganism used, but any of the culture media used for incubating ordinary microorganisms may be employed. That is, starch, dextrin, sucrose, lactose, maltose or glycerin, may be employed as the carbon source, and peptone, meat extract, yeast or its extract, soya bean powder, cornsteep liquor, gluten, urea, ammonium salts or nitrates, may conveniently be employed as the nitrogen source. If necessary, inorganic salts of such metals as magnesium, calcium, potassium, sodium and a trace of copper, iron, manganese or cobalt may be added, or the incubation may be conducted in a medium already containing trace elements. In some cases, ribonucleic acid or oligoribonucleotide itself may be utilised as a nutrient source.

The reaction proceeds by contact between ribonucleic acid or oligoribonucleotide and the microorganisms or their enzyme system. When living cells of the microorganism are used, ribonucleic acid or oligoribonucleotide may be added to the medium at a stage of the incubation, or the microorganism may be incubated on a medium containing the starting material.

When the enzyme system of the microorganism is used, the culture filtrate or cell suspension of the microorganism, or its extracted enzyme, may be brought into contact with the starting material, with or without using a convenient solvent to first dissolve the starting material. If the culture filtrate or cell suspension is employed, a solvent need not necessarily be used in most cases.

When a solvent is used, water or an aqueous solvent serves for the purpose and the latter may, for example, be an aqueous solution of a salt, such as a buffer solution.

The reaction should be conducted at a pH most suitable for the exhibition of enzymic activity even when the extracted enzyme is used, as in the case when living cells are used. Such a pH is between 4 and 10. The reaction temperature should not be such as to weaken the enzymic activity too much or destroy the enzyme system. The temperature is variable according to the kind of microorganism used and other conditions, and may ordinarily be found in the range between 30 and 45°C. The reaction time is also variable in accordance with such conditions as the kind of microorganism, enzyme or medium, concentration of the material in the medium and reaction temperature, but the reaction should be continued until the desired product is produced in the highest yield. The reaction conditions may preferably be adjusted so that 15 to 40 hours is required for producing the desired product in the highest yield.

In the processes of this invention, an accelerator may be added to the reaction system to stimulate the enzyme activity. Such an agent is, for example, a magnesium salt.

In most cases, however, phosphomonoesterase exists in the enzyme system of the microorganisms and therefore the reaction would not stop at 5'-nucleotides but would proceed further to nucleosides. In such a case, the product may be a mixture composed of the nucleosides such as adenosine, guanosine, cytidine, uridine, inosine or xanthosine.

Thus, the invention further provides a process for preparing one or more nucleosides, which comprises bringing a ribonucleic acid and/or oligoribonucleotide into contact, at a pH between 4 and 10, with phosphodiesterase and phosphomonoesterase produced by a microorganism belonging to one of the genera *Streptomyces*, *Helminthosporium*, *Gliomastix*, *Verticillium* or *Fusarium*.

In order to obtain 5'-nucleotides alone phosphomonoesterase must be removed from the enzyme system by a purification process, or the action of phosphomonoesterase should be obstructed by a suitable method. In order to obstruct the action of phosphomonoesterase, a phosphomonoesterase inhibitor may be added to the enzyme system. Examples of such agents are phosphates, arsenates, cyanates,

cysteine, glutamic acid, ethylenediamine tetra-acetic acid, zinc ions and cupric ions. When deaminase co-exists with phosphodiesterase, deaminated compounds may contaminate the product in such a case. Such a reaction, however, may preferably be utilised for obtaining deamination products such as inosinic acid.

When the phosphomonoesterase activity of the enzyme system is very high, use of a phosphomonoesterase inhibitor allows mainly 5'-nucleotides to be formed, while non-use of the said inhibitor allows a mixture comprising mainly nucleosides to be formed due to hydrolysis by the phosphomonoesterase. In both cases, some oligonucleotides are usually admixed with the product. Even when a phosphomonoesterase inhibitor is used, admixture of nucleosides in the product is difficult to avoid or alternatively admixture of deamino compounds in the product may occur. Therefore it is convenient that the reaction is brought to an end at the time when the concentration of the desired compound in the reaction mixture becomes the highest, the progress of the hydrolysis being followed by measuring the concentration. The measurement of the concentration may be conducted utilising a known method, such as an enzymic process, paper-electrophoresis or paper-chromatography.

From the mixture thus produced, which contains chiefly 5'-nucleotides with or without nucleosides, the desired substances can be isolated, utilising differences in physico-chemical properties between impurities and the desired substances. Differences in solubility, distribution coefficient between two solvents, absorbability, dialysability and precipitability are among the physico-chemical properties which may be utilised for the purpose. Further, 5'-nucleotides may conveniently be collected as their organic or inorganic salts, for instance, as salts with barium, calcium, potassium, ammonium, aminoacids, cyclohexylamine or brucine.

The process for separating and purifying each component of the mixture may be conducted by a method which is itself known, such as chromatography. An example of such a method is explained hereunder:

Insoluble impurities are first removed from the mixture by filtration and then the active substances are adsorbed on an activated charcoal. The adsorbed substances are eluted with a convenient solvent, such as dilute hydrochloric acid, dilute ammonia or alcohol. The ultraviolet absorption of each fraction is then measured, and each fraction containing a component is collected and concentrated. If the product is collected as its barium salt, the subsequent purification can be conducted more conveniently.

The above process is an example of column chromatography on activated charcoal, and other processes such as ion-exchange chromato-



graphy on ion-exchangers or counter-current distribution processes can also be utilised for the same purpose.

The following Examples further illustrate the present invention, Example 3 being an Example where the phosphomonoesterase formation is not inhibited, so that hydrolysis to the nucleoside takes place. In the Examples, the temperatures are all in degrees Centigrade and are all uncorrected, the percentages are all by weight, and the abbreviations AMP, GMP, UMP, CMP and IMP mean adenosine monophosphate, guanosine monophosphate, uridine monophosphate, cytidine monophosphate, and inosine monophosphate, respectively. The nucleic acid used for the starting materials of the Examples is that produced from yeast by a known process, except when otherwise noted. According to this process, yeast was extracted by heating with a neutral or weakly alkaline saline solution (10%) and precipitated from the extract with dilute hydrochloric acid or ethanol. The resulting ribonucleic acid contains, in addition, oligoribonucleotides. The names of culture collections abbreviated as IFO, NRRL, ATCC and CBS are Institute for Fermentation, Osaka, Japan, Northern Utilization Research Branch of United States Department of Agriculture, Peoria, Illinois, United States of America, American Type Culture Collection, Washington, D.C. United States of America, and Centraalbureau voor Schimmelcultures, Baarn, Holland, respectively.

EXAMPLE 1.

A strain of *Streptomyces albogriseolus* Benedict, Shotwell et Pridham is incubated for 24 hours at a temperature of 28° on an aqueous medium, whose pH is 7.0, composed of 5.0% of soluble starch, 3.0% of cornsteep liquor, 0.1% of ammonium sulphate, 0.05% of hydrous magnesium sulphate, 0.5% of peptone and 0.2% of calcium carbonate. On 100 litres of the same medium in a tank is inoculated 500 ml. of the culture described above, then the incubation is conducted at a temperature of 28° for 30 hours. A suspension of 1 kg. of ribonucleic acid in 200 litres of water is adjusted to pH 7.8 with a solution of sodium hydroxide. To the solution is added 100 litres of the culture filtrate of the above culture and sodium arsenate is then added to give a concentration in the solution of 10 millimole per litre, whereafter the pH is adjusted to 8.0, with sodium hydroxide. After the addition of 500 g. of toluene for the purpose of antiseptis, the hydrolysis is conducted at a temperature of 37° for 16 hours, keeping the pH at 7.5—8.0 with sodium hydroxide. The reaction mixture is filtered with a filtering supplement, and the pH of the filtrate is lowered to 2.5. After the product in the filtrate has been adsorbed on activated charcoal, it is eluted with 1.5% aqueous ammonia. Nucleo-

tides in the eluate are separated with the aid of a "DOWEX—1" ion exchange resin, (the word "DOWEX" is a Registered Trade Mark) and the fraction containing 5'-AMP is collected. The fraction containing 5'-AMP is deaminated with sodium nitrite to obtain 100 g. of 5'-IMP. From the other fractions, 5'-GMP, 5'-UMP and 5'-CMP are collected, and the respective yields are 55 g., 70 g. and 78 g.

The strain of *Streptomyces albogriseolus* is distributed from NRRL under the number of B—1305, through the Institute for Applied Microbiology of Tokyo University and the Faculty of Agriculture, University of Tokyo.

EXAMPLE 2.

A strain of *Streptomyces aureus* (Waksman et Curtis) Waksman et Henrici is incubated with shaking for 24 hours at a temperature of meat extract, 0.05% of hydrated magnesium sulphate and 0.5% of sodium chloride. On 28° in an aqueous medium, whose pH is 7.0, sulphate and 0.5% of sodium chloride. On 100 litres of a medium having the same composition as the above is inoculated 500 ml. of the above seed culture, and the incubation is conducted in a tank for 45 hours at a temperature of 28°.

To a suspension of 1 kg. of ribonucleic acid in 200 litres of water are added 100 litres of the culture filtrate of the above culture, an aqueous solution of sodium hydroxide to make the pH of the mixture 7.8, and sodium arsenate to give a concentration in the mixture of 10 millimole per litre, successively. After the pH of the mixture is adjusted to 8.0, with sodium hydroxide, 500 g. of toluene is added for the purpose of antiseptis. The hydrolysis is then conducted for 20 hours at a temperature of 37°, keeping the pH at 7.5—8.0 with sodium hydroxide. The reaction mixture is filtered with a filtering supplement, and the pH of the filtrate is lowered to 3.0 with acid. After the product in the filtrate has been adsorbed on activated charcoal, it is eluted with 1.5% aqueous ammonia. From the eluate there are separated with the aid of a "DOWEX—1" ion-exchange resin 5'-IMP, 5'-UMP, 5'-GMP and 5'-CMP, their respective yields being 120 g., 75 g., 90 g. and 95 g.

The strain of *Streptomyces aureus* used in this Example is designated as Ogata strain No. 1031 and is deposited at IFO under the accession number IFO-3303, and also at ATCC under the accession number ATCC-13404.

EXAMPLE 3.

A strain of *Streptomyces viridochromogenes* (Krainsky) Waksman et Henrici is incubated at a temperature of 28° for 24 hours on an aqueous medium, whose pH is 7.0, composed of 3.0% of soluble starch, 1.0% of peptone, 0.5% of meat extract, 0.05% of hydrated magnesium sulphate and 0.1% of potassium phos-

28° in an aqueous medium, whose pH is 7.0, composed of 5.0% of soluble starch, 1.0% of

phate. On 100 litres of a medium having the same composition as the above is inoculated 500 ml. of the above seed culture and the incubation is conducted in a tank for 60 hours at a temperature of 28°.

To a suspension of 1 kg. of ribonucleic acid in 200 litres of water is added an aqueous solution of sodium hydroxide to bring the pH of the mixture to 7.8, followed by 100 litres of the culture filtrate of the above culture. The hydrolysis is then conducted at a temperature of 37° for 30 hours while the pH is kept at 8.0 by adding sodium hydroxide. As the culture filtrate contains phosphomonoesterase in high activity, the ribonucleic acid is almost quantitatively hydrolysed into nucleosides. From the hydrolysate are collected adenosine, guanosine, cytidine and uridine, and their respective yields are 150 g., 110 g., 130 g., and 140 g.

The strain *Streptomyces viridochromogenes* used in this Example is a strain distributed from CBS. The strain was originally sent to the said culture collection from Dr. S. A. Waksman, Rutgers University, New Brunswick, New Jersey, United States of America.

EXAMPLE 4.

A strain of *Streptomyces purpureus* Lindenbein was incubated at a temperature of 28° for 15 hours on an aqueous medium, whose pH is 7.0, composed of 3.0% of soluble starch, 1.0% of peptone, 0.5% of yeast extract, 0.05% of hydrated magnesium sulphate, 0.05% of calcium carbonate and 0.1% of sodium chloride. To 100 litres of the culture filtrate of the above culture is added 1 kg. of ribonucleic acid, followed by sodium arsenate to give a concentration in the mixture of 10 millimole per litre. The hydrolysis is then conducted at a temperature of 37° for 20 hours while the pH is kept at 7.5—8.0 with sodium hydroxide. From the hydrolysate are collected 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP, and their respective yields are 75 g., 70 g., 80 g., and 75 g. Both nucleosides and oligonucleotides are collected from what remains.

The strain of *Streptomyces purpureus* used in this Example is a strain stored at IFO under the accession number of IFO-3389, and was originally distributed from NRLL under the number of B-1454, via the National Institute of Health, Bethesda, Maryland, United States of America.

EXAMPLE 5.

A strain of *Streptomyces coelicolor* (Müller) Waksman et Henrici is incubated in a tank for 15 hours at a temperature of 28° in an aqueous medium, whose pH is 7.0, composed of 5.0% of soluble starch, 3.0% of cornsteep liquor, 1.0% of peptone, 0.05% of ammonium sulphate, 0.2% of calcium carbonate and 0.2% of sodium chloride. To 100 litres of the

culture filtrate of the above culture is added 1 kg. of ribonucleic acid, and the mixture is left standing for 16 hours at a temperature of 37°. From the hydrolysate are collected 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP, and their respective yields are 55 g., 30 g., 45 g., and 48 g. A part of the hydrolysate is collected as adenosine, guanosine, cytidine and uridine, as a result of the dephosphorilation. In this Example, the microorganism itself acts as a phosphomonoesterase inhibitor.

The strain of *Streptomyces coelicolor* is a strain stored in IFO under the accession number IFO-3807, and was filed at ATCC under the accession number of ATCC-13405.

EXAMPLE 6.

A strain of *Helminthosporium sigmoideum* var. *irregulare* Cralley et Tullis is incubated for 45 hours at a temperature of 28° in a tank in an aqueous medium, whose pH is 7.0, composed of 5.0% of glucose, 1.0% of peptone, 1.0 % of soya bean powder, 0.05% of hydrated magnesium sulphate, 0.1% of potassium phosphite, and 0.2% of sodium chloride. To a suspension of 1 kg. of ribonucleic acid in 200 litres of water is added 100 litres of the culture filtrate of the above culture, and sodium arsenate is added to a concentration in the mixture of 10 millimoles per litre. The hydrolysis is then conducted at a temperature of 37° for 16 hours while the pH is kept at 7.5—8.0 with sodium hydroxide. From the hydrolysate there is collected 5'-AMP, 5'-GMP, 5'-UMP and 5'-CMP, and their respective yields are 70 g., 50 g., 65 g. and 65 g. Further, adenosine, guanosine, cytidine and uridine are collected from a part of the remainder as the result of dephosphorilation.

The strain of *Helminthosporium sigmoideum* var. *irregulare* used in this Example is deposited at IFO under the accession number IFO-5273, and was filed with ATCC under the accession number ATCC-13406.

EXAMPLE 7.

A strain of *Streptomyces coelicolor* is incubated for 15 hours at a temperature of 28° in a tank on an aqueous medium, whose pH is 7.0, composed of 5.0% of soluble starch, 3.0% of cornsteep liquor, 1.0% of peptone, 0.05% of hydrated magnesium sulphate, 0.1% of ammonium sulphate, 0.2% of calcium carbonate and 0.2% of sodium chloride.

To an aqueous extract, which was made from 10 kg. of yeast and 150 litres of water at a pH of 7—9 with heating, are added 35 litres of the culture filtrate of the above culture, followed by sodium arsenate to a concentration in the mixture of 10 millimoles per litre. The hydrolysis is then conducted at a temperature of 37° for 20 hours while the pH is kept at 7.5—8.0 with sodium hydroxide. From the hydrolysate are collected 5'-AMP, 5'-GMP, 5'-CMP and 5'-UMP,

and their respective yields are 110 g., 70 g., 90 g., and 80 g. Further, nucleosides and oligonucleotides are collected from what remains.

- 5 The strain of *Streptomyces coelicolor* used in this Example is the same strain as used in Example 5.

WHAT WE CLAIM IS:—

- 10 1. A process for the production of 5'-nucleotides, which comprises contacting one or more ribonucleic acids and/or oligoribonucleotides at a pH between 4 and 10 with living cells of, or an enzyme system produced by, a microorganism belonging to the genus
- 15 *Streptomyces* in the absence of phosphomonoesterase or in the presence of a phosphomonoesterase inhibitor, (which inhibitor may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced.

- 20 2. A process for the production of 5'-nucleotides, which comprises contacting one or more ribonucleic acids and/or oligoribonucleotides at a pH between 4 and 10 with living cells of, or an enzyme system produced by, a microorganism belonging to the class *Fungi imperfecti* in the absence of phosphomonoesterase or in the presence of a phosphomonoesterase inhibitor, (which inhibitor may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced.

3. A process as claimed in claim 1 or claim 2, wherein the ribonucleic acid comprises yeast ribonucleic acid.

- 35 4. A process as claimed in any of claims 1 to 3, wherein deaminase is present during the contacting.

- 40 5. A process as claimed in claim 2, wherein the enzyme system is produced by a microorganism belonging to the genus *Helminthosporium*.

6. A process as claimed in claim 2, wherein the enzyme system is produced by a microorganism belonging to the genus *Gliomastix*.

- 45 7. A process as claimed in claim 2, wherein the enzyme system is produced by a microorganism belonging to the genus *Verticillium*.

8. A process as claimed in claim 2, wherein the enzyme system is produced by a microorganism belonging to the genus *Fusarium*.

- 50 9. A process as claimed in claim 1 or claim 2, wherein the phosphomonoesterase inhibitor is ethylene diamine tetraacetic acid, a phosphate or an arsenate.

- 55 10. A process as claimed in any of the preceding claims, wherein each component of the product is separated by column chromatography on activated charcoal, each component being collected from the chromatographic eluate by concentration.
- 60

11. A process as claimed in claim 10, wherein the components are collected as their barium salts.

12. A process as claimed in claim 10, wherein the components are collected as their calcium salts.

13. A process as claimed in claim 10, wherein the components are collected as their cyclohexylamine salts.

14. A process as claimed in any of the preceding claims, wherein the microorganism is incubated on a nutrient medium containing the ribonucleic acid and/or oligoribonucleotide.

15. A process as claimed in any of claims 1 to 13, wherein the ribonucleic acid and/or oligoribonucleotide is admixed with a culture filtrate of the microorganism.

16. A process as claimed in any of claims 1 to 13, wherein the ribonucleic acid and/or ribonucleotide is admixed with a cell suspension of the microorganism.

17. A process as claimed in any of claims 1 to 13, wherein the ribonucleotide and/or ribonucleic acid is treated with an extracted enzyme of the microorganism in an aqueous medium.

18. A process for the production of 5'-nucleotides, substantially as described with reference to any of Examples 1, 2, 4, 5, 6 or 7.

19. 5'-Nucleotides when produced by the process claimed in any of the preceding claims.

20. A seasoning which comprises a 5'-nucleotide as claimed in claim 19 and an inert carrier.

21. A process for seasoning foodstuffs, which comprises adding to said foodstuff one or more 5'-nucleotides produced by the process claimed in claim 1.

22. A process for hydrolysing ribonucleic acids and/or oligoribonucleotides into one or more nucleosides, which comprises bringing the ribonucleic acid and/or oligoribonucleotide into contact, at a pH between 4 and 10, with phosphodiesterase and phosphomonoesterase produced by a microorganism belonging to the genus *Streptomyces*.

23. A process for hydrolysing ribonucleic acid and/or oligoribonucleotides into one or more nucleosides, which comprises bringing the ribonucleic acid and/or oligoribonucleotide into contact, at a pH between 4 and 10, with phosphodiesterase and phosphomonoesterase produced by a microorganism belonging to the genus *Helminthosporium*, genus *Gliomastix*, genus *Verticillium* or genus *Fusarium*.

24. A process as claimed in claim 22, substantially as described with reference to Example 3.

25. Nucleosides when produced by a process as claimed in claims 23 or 24.



and their respective yields are 110 g., 70 g., 90 g., and 80 g. Further, nucleosides and oligonucleotides are collected from what remains.

- 5 The strain of *Streptomyces coelicolor* used in this Example is the same strain as used in Example 5.

WHAT WE CLAIM IS:—

- 10 1. A process for the production of 5'-nucleotides, which comprises contacting one or more ribonucleic acids and/or oligoribonucleotides at a pH between 4 and 10 with living cells of, or an enzyme system produced by, a microorganism belonging to the genus
15 *Streptomyces* in the absence of phosphomonoesterase or in the presence of a phosphomonoesterase inhibitor, (which inhibitor may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced.
- 20 2. A process for the production of 5'-nucleotides, which comprises contacting one or more ribonucleic acids and/or oligoribonucleotides at a pH between 4 and 10 with living cells of, or an enzyme system produced by, a microorganism belonging to the
25 class *Fungi imperfecti* in the absence of phosphomonoesterase or in the presence of a phosphomonoesterase inhibitor, (which inhibitor may be the microorganism itself), and separating the 5'-nucleotide or nucleotides produced.
- 30 3. A process as claimed in claim 1 or claim 2, wherein the ribonucleic acid comprises yeast ribonucleic acid.
- 35 4. A process as claimed in any of claims 1 to 3, wherein deaminase is present during the contacting.
5. A process as claimed in claim 2, wherein the enzyme system is produced by a micro-
40 organism belonging to the genus *Helminthosporium*.
6. A process as claimed in claim 2, wherein the enzyme system is produced by a micro-
45 organism belonging to the genus *Gliomastix*.
7. A process as claimed in claim 2, wherein the enzyme system is produced by a micro-
50 organism belonging to the genus *Verticillium*.
8. A process as claimed in claim 2, wherein the enzyme system is produced by a micro-
55 organism belonging to the genus *Fusarium*.
9. A process as claimed in claim 1 or claim 2, wherein the phosphomonoesterase inhibitor is ethylene diamine tetraacetic acid, a phosphate or an arsenate.
- 60 10. A process as claimed in any of the preceding claims, wherein each component of the product is separated by column chromatography on activated charcoal, each component being collected from the chromatographic eluate by concentration.

11. A process as claimed in claim 10, wherein the components are collected as their barium salts.

12. A process as claimed in claim 10, wherein the components are collected as their
65 calcium salts.

13. A process as claimed in claim 10, wherein the components are collected as their cyclohexylamine salts.

14. A process as claimed in any of the preceding claims, wherein the microorganism is incubated on a nutrient medium containing the ribonucleic acid and/or oligoribonucleotide.
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15. A process as claimed in any of claims 1 to 13, wherein the ribonucleic acid and/or oligoribonucleotide is admixed with a culture filtrate of the microorganism.
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16. A process as claimed in any of claims 1 to 13, wherein the ribonucleic acid and/or ribonucleotide is admixed with a cell suspension of the microorganism.
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17. A process as claimed in any of claims 1 to 13, wherein the ribonucleotide and/or ribonucleic acid is treated with an extracted enzyme of the microorganism in an aqueous medium.
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18. A process for the production of 5'-nucleotides, substantially as described with reference to any of Examples 1, 2, 4, 5, 6 or 7.

19. 5'-Nucleotides when produced by the process claimed in any of the preceding claims.
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20. A seasoning which comprises a 5'-nucleotide as claimed in claim 19 and an inert carrier.

21. A process for seasoning foodstuffs, which comprises adding to said foodstuff one or more 5'-nucleotides produced by the process claimed in claim 1.
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22. A process for hydrolysing ribonucleic acids and/or oligoribonucleotides into one or more nucleosides, which comprises bringing the ribonucleic acid and/or oligoribonucleotide into contact, at a pH between 4 and 10, with phosphodiesterase and phosphomonoesterase produced by a microorganism belonging to the genus *Streptomyces*.
100

23. A process for hydrolysing ribonucleic acid and/or oligoribonucleotides into one or more nucleosides, which comprises bringing the ribonucleic acid and/or oligoribonucleotide into contact, at a pH between 4 and 10, with phosphodiesterase and phosphomonoesterase produced by a microorganism belonging to the genus *Helminthosporium*, genus *Gliomastix*, genus *Verticillium* or genus *Fusarium*.
105

24. A process as claimed in claim 22, substantially as described with reference to Example 3.
110

25. Nucleosides when produced by a process as claimed in claims 23 or 24.
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