

Patented Sept. 17, 1940

2,215,233

# UNITED STATES PATENT OFFICE

2,215,233

## IRON COMPOUND OF NUCLEOTIDES AND THEIR ORGANIC HYDROLYTIC DECOMPOSITION PRODUCTS AND METHOD OF MAKING SAME

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No Drawing. Application November 13, 1937,  
Serial No. 174,484

6 Claims. (Cl. 167-68)

This invention relates to organic compounds of iron and more particularly to therapeutic preparations containing iron compounds of nucleotides and their organic hydrolytic decomposition products which contain a pyrimidine ring in their molecule. The present application is a continuation in part of my copending applications Serial No. 650,137, filed January 4, 1933, now Patent No. 2,115,751 of May 3, 1938, and Serial Nos. 696,388 and 696,390, filed November 2, 1933, the latter having issued as Patent No. 2,098,976 of Nov. 16, 1937.

It is an object of the invention to provide a therapeutic preparation containing a high proportion of iron and possessing desirable therapeutic activity when administered into the animal organism.

A further object of the invention is to provide a method for producing iron compounds of nucleotides or their organic hydrolytic decomposition products which contain a pyrimidine ring, such as the nucleosides, or the purine and pyrimidine bases derived therefrom.

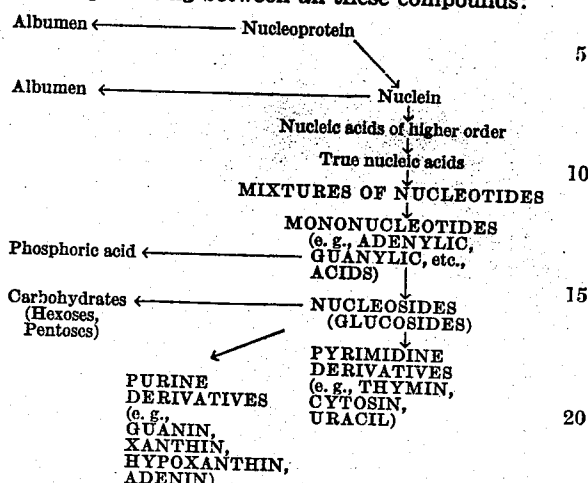
Another object of this invention is to provide a method whereby insoluble or difficultly soluble compounds of iron with nucleotides or their organic hydrolytic decomposition products which contain a pyrimidine ring, such as the nucleosides, or the purine and pyrimidine bases derived therefrom may be brought into solution to enable them to be administered internally in high concentration, either by injection or per os.

Under the expression "nucleotides and their organic hydrolytic decomposition products which contain a pyrimidine ring, such as the nucleosides, or the purine and pyrimidine bases derived therefrom" are to be understood compounds which are obtained or are obtainable from nucleoproteins by hydrolysis. The nucleotides are composed of "nucleosides" and phosphoric acid and are acids; they are decomposed by the action of hot water, ferments, acids or alkalis. The mononucleotides can be isolated from pancreas and from extract of meat as well as from yeast and also other substances in any known manner.

The nucleosides which, in combination with phosphoric acid, form the nucleotides, are glucosides of various purine and pyrimidine bases. They are obtained by the action of ferments and are finally decomposed into the various "purine" and "pyrimidine" bases, of which the following may be mentioned: thymine, cytosine, uracil, adenine and guanine. These final hydrolytic decomposition products have also been produced directly from nucleoproteins, nucleins and nucleotides by the action of acids or alkalis.

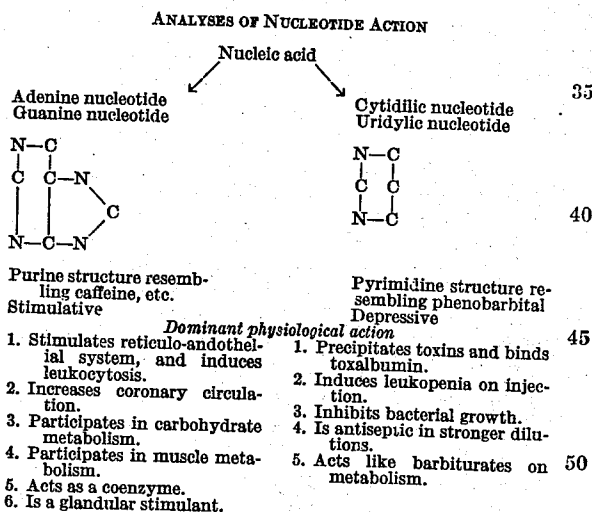
As the chemical constitution and the nomenclature of the more complex organic compounds employed in the process of this invention are still in a state of considerable confusion, the follow-

ing table is presented to give a clearer picture of the scope of the invention and of the relationship existing between all these compounds:



Of course, the invention comprises not only the compounds as obtained by hydrolytic decomposition of nucleoproteins, but also those compounds which are produced synthetically, i. e., in the reverse order of the table.

If we group the nucleotides in accordance with their physiologic action, we get a schematic arrangement as follows:



It will readily be seen that the individual nucleotides are radically different from the larger molecule, the nucleic acid, not merely in chemical constitution but in specific physiologic actions. It will be noted that the purine nucleotides on the one hand, and the pyrimidine nucleotides on the other, while they both contain a

pyrimidine ring in their molecules and may thus both be regarded as pyrimidine ring-containing compounds, have opposed physiologic effects; in fact, in certain properties the purine nucleotides are more or less opposites of each other, and the same is true of the pyrimidine nucleotides. I have found that advantage can be taken of the specific physiologic actions of the different nucleotides and of their pyrimidine-ring containing decomposition products by combining them with iron, either ferrous or ferric, the resulting compounds, probably because of their close relationship to substances naturally present in the animal tissues, representing also excellent media for the introduction of iron into the organism.

The more specific virtue of the iron salts of the purine nucleotides lies in their ability to elevate the whole blood picture; that is, the haemoglobin content and the red blood cell (erythrocyte) and white blood cell (leucocyte) counts are increased. This is unique, since the action of known therapeutic iron salts is to elevate only the haemoglobin and red blood cell count. The supplementary action of the purine nucleotide portion of the salt elevates the white blood cell count. In addition, there is a remarkable synergistic action between the adenylic nucleotides and the haemoglobin and red blood cells. Thus there is always a direct ratio between the adenylic acid content of the blood and the haemoglobin and the red blood cell count. When the adenylic acid content of the blood is high, there is a high haemoglobin and red cell count. Inversely, where the haemoglobin and red cell count is low, there is a low adenylic acid content. Thus ferrous adenylate or guanylate presents a highly desirable and unique therapeutic preparation.

Another important advantage resulting from the use of these combinations of iron and nucleotides or their organic hydrolytic decomposition products therapeutically is that they have a very great stimulating effect upon the reticulo-endothelial system, so that they are of great value in the treatment of diseases caused by deficiencies of this system, such as agranulocytosis, benzene poisoning, X-ray poisoning and various leucopenias incident to exhaustion of the reticulo-endothelial systems.

The clinical application of this product has been demonstrated and the results obtained are published in *Annals of Internal Medicine*, vol. 9, No. 11, May 1936.

For the purpose of illustrating my invention in greater detail and by way of example, I will describe the preparation of the combination of iron with adenylic nucleotide.

Ferrous adenylate may be made in the following manner. 25 grams of adenylic acid are suspended in approximately 300 cc. of water and neutralized with ammonia. 50 grams of ferrous ammonium sulphate are dissolved in about 200 cc. of water. In order to be certain that all the iron is in the ferrous state, a few drops of sulphuric acid are added and also a small amount of iron filings. This solution is then agitated, filtered and added to the solution of adenylic acid. A precipitate of ferrous adenylate or guanylate is obtained. Approximately 500 cc. of alcohol are now added. The mixture is then allowed to stand for a couple of hours during which time coagulation and settling takes place. The ferrous adenylate is then filtered off with the aid of such. The ferrous adenylate is sludged up in about 300 cc. of water, an equal quantity

of alcohol is added, and the mixture filtered. The precipitate is then dried in a desiccator.

The ferrous adenylate may be used in tablet form or dissolved with the addition of sodium citrate and put in ampoule form for injection or in bottles for administration per os.

In place of the ferrous ammonium sulphate, ferrous chloride may be used, in which case it is desirable to employ sodium adenylate (or guanylate, etc.) as sodium chloride is then produced which need not be separated from the iron nucleotide.

Other compounds than the adenylate, for example, the guanylate cytidilate, and uridyate may be produced in a similar manner from the corresponding nucleotides. As starting material may be used the alkali-metal salts of the purine or pyrimidine nucleotides or their organic hydrolytic decomposition products which contain a pyrimidine ring and are capable of combining with iron. Separation of the crude precipitate, as well as of the purified compound, may be carried out in any suitable manner, by filtering, centrifuging, pressing through a filter press, decanting or in any other suitable way. Suspension of the crude product in water, in order to purify it, may also be accomplished by extracting the precipitate in suitable extraction apparatus, by macerating or by any other suitable method, while stirring or shaking at ordinary or elevated temperature. Extraction is especially advisable in the case of the more difficultly soluble compounds of mononucleotides.

Instead of sodium citrate, other salt solutions, such as potassium acetate or citrate, sodium chloride, sodium nitrate, sodium acetate, ammonia citrate and others may be used for solubilizing insoluble or difficultly soluble compounds.

As a precipitating agent one may employ benzene, ether or any other organic liquid in which the iron compound produced is insoluble.

The drying may be effected at ordinary or elevated temperature, in a vacuum or by means of an air current or in any other suitable manner, care being taken that the conditions are not such that the iron compound is decomposed.

The reaction products of the organic and inorganic iron compounds on the one hand and nucleoproteins, nucleotides and their hydrolytic decomposition products on the other hand can be purified in another manner than by redissolving or suspending and re-precipitating, e. g., by recrystallization, or by any other suitable method.

While I prefer to produce the ferrous compounds of the organic substances above mentioned, the ferric compounds are also of value; and generally, although not necessarily, a certain amount of the ferric will accompany the ferrous compound or compounds.

The individual, isolated iron nucleotides may be used singly, or they may be used in the form of two isolated nucleotides, especially where it is desired to suppress one or more actions of a nucleotide, in which case a nucleotide having an opposite action is used simultaneously. Thus, two pyrimidine or two purine nucleotides may be used simultaneously; and for certain purposes an isolated pyrimidine iron compound may with advantage be mixed with an isolated purine iron compound.

As already indicated, the iron compounds above described may be administered either orally or subcutaneously. In the latter mode, intramuscular injection is preferred; the dosage is about  $\frac{1}{2}$  to 2 grains of the iron compound by injection

daily or every two days; while orally from 5 to 10 grains or more may be administered as often as three times a day, depending on the tolerance of the stomach and requirements of the patient.

5 It will be understood that the invention is not limited to the specific examples given, as modifications may be made by those skilled in the art in accordance with the principles herein set forth.

10 Thus the free nucleotides may be reacted with iron compounds, including basic iron compounds to produce the iron nucleotides.

I claim:

1. A medicinal preparation for the treatment of diseases by application to the human or animal body comprising a solubilized iron compound of isolated adenylic acid.

15 2. A medicinal preparation for the treatment of diseases by application to the human or animal body comprising the isolated adenylic acid compound of ferrous iron.

20 3. A medicinal preparation for the treatment of diseases by application to the human or animal body comprising an iron compound of an isolated mononucleotide and a solubilizing agent therefor comprising an alkali metal salt of an organic acid.

25 4. A therapeutic preparation comprising the combination of ferrous iron and a compound

selected from the group consisting of mononucleotides and their organic hydrolytic decomposition products which contain a pyrimidine ring in their molecule and which are capable of combining with iron, said combination being soluble in water and in organic salt solutions. 5

5. A method of producing iron compounds of mononucleotides and their organic hydrolytic decomposition products which contain a pyrimidine ring in their molecule, consisting in reacting a compound selected from the group of compounds consisting of mononucleotides and their organic hydrolytic decomposition products which contain a pyrimidine ring in their molecule, with an aqueous solution of an iron salt, separating the precipitated reaction product from the mother liquor, dissolving said precipitate in an aqueous solution with the aid of a salt of the group consisting of sodium citrate, ammonium citrate, sodium chloride, sodium nitrate, and the corresponding potassium salts. 10 15 20

6. A medicinal preparation comprising the reaction product of a ferrous compound and a member of the group consisting of free and neutralized adenylic acid. 25

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