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ALKALI METAL SALTS OF ADENYLIC ACID

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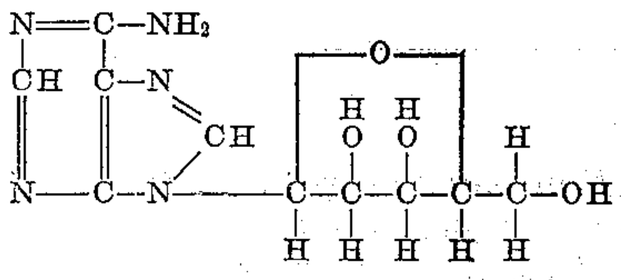
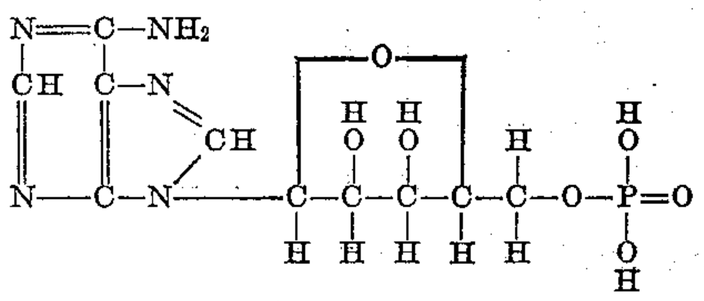
This invention relates to alkali metal salts of adenylic acid.

The so-called adenylic acid system includes a series of rather complicated compounds which are combinations of the base adenine (6-amino purine), the pentose d-ribose, and phosphoric acid, among others. The combination of adenine with ribose linked in glycosidic union at the 9-position of the base constitutes the substance known as adenosine, which has the following formula:

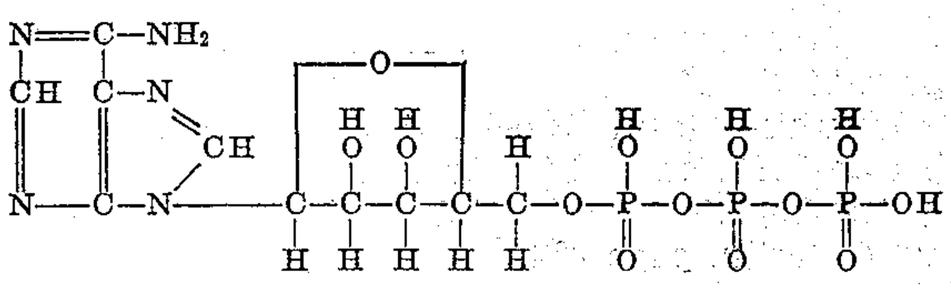


The mono-phosphoric derivative of adenosine (adenosine-5-mono-phosphoric acid) is designated herein as adenylic acid. It has the following formula:



It may be prepared from yeast or muscle and for the purpose of describing this invention, the adenylic acid referred to is that of the above formula irrespective of its origin.

Adenylic acid is one of three related coenzyme-active substances constituting the adenylic acid system, and the other members are more highly phosphorylated derivatives, namely the di- and tri-phosphoric acid compounds. For example, adenosine tri-phosphoric acid (or adenosine pyrophosphoric acid or adenylpyrophosphoric acid, as it is sometimes termed) has been assigned the following formula:



Some references give other formulas, but the above formula represents the best current information on the structure of the compound.

The adenylic acid system in the body appears to constitute a mobile equilibrium which functions as a carrier for phosphoric acid, and is

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essential to carbohydrate metabolism. The lower phosphate esters of adenosine act as phosphoric acid acceptors, and the more highly phosphorylated derivatives act as donators of the acid.

It is known that muscle contains adenosine tri-phosphoric acid. Adenylic acid as such is not contained in animal tissue. I am aware that the alkali metal salts of adenosine tri-phosphoric acid have been proposed for therapeutical purposes (U. S. Patent No. 1,978,881), and that adenylic acid as such, both from yeast and muscle sources, has been prepared, as well as adenosine tri-phosphoric acid and the alkali metal salts of the latter as referred to above.

I have discovered that an alkali metal salt of adenylic acid is advantageous for therapeutic administration. Although the body does not contain adenylic acid, it is believed to use this as a source for the formation of the muscle component, namely adenosine tri-phosphoric acid. When administered as the alkali metal adenylate, the adenylic acid is utilized in a slow and steady way and therefore is capable of giving a more sustained effect than is possible with the administration of adenosine tri-phosphoric acid or its alkali metal salts.

Furthermore, the alkali metal salts of adenylic acid are exceptionally stable as contrasted with the adenosine tri-phosphoric acid and its salts. The sodium salts of the latter decompose in time even when maintained in a normally dry state. In contrast, the alkali metal salts of adenylic acid are remarkably stable, not only in a dry state but even in solution. The latter property permits the alkali metal salts of adenylic acid to be packaged in aqueous solution in sterilized ampule form for intra-muscular administration, with the knowledge that the activity will not deteriorate upon storage of the solution.

The alkali metal salts of adenylic acid in the dry form, in addition to being stable, are extremely soluble as compared with the salts heretofore known, and may be administered orally during which time they can be absorbed through the mucous membrane.

The alkali metal salts of adenylic acid may be prepared from the adenosine tri-phosphoric acid naturally occurring in muscle or from yeast in accordance with known hydrolysis and separation techniques.

For example, adenosine in a buffered phosphate solution may be reacted with yeast or a yeast plasmolysate to produce adenosine tri-phosphoric acid and cozymase in addition to adenylic acid. In order to convert the higher

phosphoric acid compounds into the adenylic acid they may be subjected to hydrolysis as described in U. S. Patent No. 2,174,475.

Another method for the preparation of adenylic acid from adenosine tri-phosphoric acid obtained from yeast is described in U. S. Patent No. 1,976,175. Preparation of adenylic acid from adenosine tri-phosphoric acid from muscle is described in U. S. Patent No. 1,977,525.

In general all of these processes precipitate the adenosine tri-phosphoric acid with an alkaline earth metal base such as barium hydroxide which is hydrolyzed in an appropriate basic solution. The second or third phosphate radicals split off during hydrolysis and are precipitated as barium phosphate leaving the barium salt of adenylic acid in solution. The latter is then precipitated as the lead salt and the lead removed as a sulfide or sulfate to leave the adenylic acid in solution.

In accordance with my invention, the adenylic acid is then neutralized with sodium hydroxide, potassium hydroxide or an equivalent base. The solution may be placed in ampules and sterilized since the adenylate alkali metal salt is stable at the high temperatures used in sterilization. Alternatively the solution may be evaporated to dryness and sodium adenylate separated as a white crystalline material. Reference to the salt contemplates it in solution or crystalline form.

It will be appreciated that the adenylate salt can exist as either a mono- or di- salt form depending upon the replacement by the metal of one or both of the hydrogens of the hydroxyl groups present in the terminal phosphoric acid radical.

Example I

1 millimol (0.347 grams) of adenylic acid (adenosine-5-monophosphoric acid) was suspended in 10 cc. of distilled water and 1 millimol (0.04 grams) of sodium hydroxide was added in the form of a 4% solution. The mixture immediately becomes a clear solution. 35 cc. of acetone was added to the solution and a granular white solid precipitated which was filtered and washed with acetone. Upon drying the product was found to be mono-sodium adenylate. It was a white crystalline solid which decomposed at a high temperature before it melted. A solution of 20 mg. per cc. of water had a pH of 5.55.

Example II

1 millimol (0.347 grams) of adenylic acid (adenosine-5-monophosphoric acid) was suspended in 10 cc. of distilled water and 2 millimols (0.08 grams of sodium hydroxide was added in the form of a 4% solution. The mixture immediately becomes a clear solution. 35 cc. of acetone was added to the solution and a granular white solid precipitated which was filtered and washed with acetone. Upon drying the product was found to be di-sodium adenylate. It was a white crystalline solid which decomposed at a high temperature before it melted. A solution of 20 mg. per cc. of water had a pH of 11.0.

Example III

The procedure described in the previous two examples was repeated except that the amount

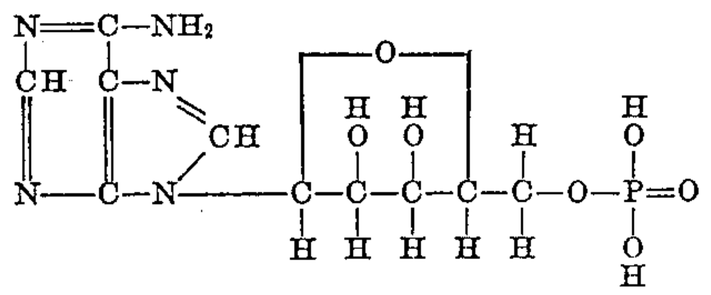
of the sodium hydroxide solution added was such that the pH of the solution was 6.5 This required 0.05 grams of sodium hydroxide. A portion of the aqueous solution was placed in ampules of 1 cc. each and sterilized. When the salt is to be packed in an aqueous solution, it is preferred that the pH be between 6 and 7 as this range is best suited for intra-muscular injection. Within this range the solution will contain both salts, the major portion being the mono-sodium salt.

Another portion of the aqueous solution was treated with acetone as in the previous examples to yield a white crystalline solid the major portion of which was the mono-sodium adenylate and the minor portion of which was the di-sodium adenylate.

The new compounds of my invention are suitable for treatment where blood analysis indicates an insufficient amount of the nucleic phosphorus compounds. Notable results have been obtained in the treatment of pruritis.

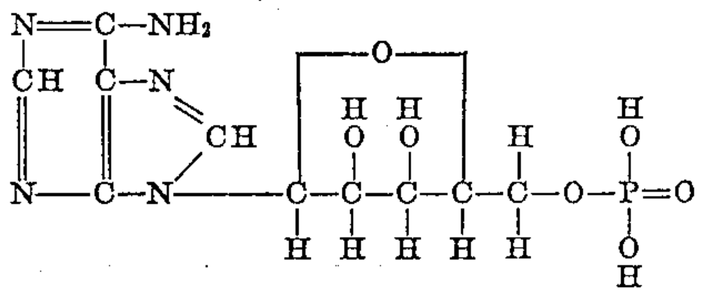
I claim:

1. A stable sterile aqueous solution of a major proportion of monosodium adenylate and a minor proportion of disodium adenylate derived from an adenylic acid having the formula:



said solution having a pH of between 5.55 and 7.

2. A sterile aqueous solution of a major proportion of monosodium adenylate and a minor proportion of disodium adenylate derived from an adenylic acid having the formula:



said solution having a pH of between 6 and 7.

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