Cl. 260

Published Apr. 20, 1943

Serial No. 257,543

ALIEN PROPERTY CUSTODIAN

MANUFACTURE OF NUCLEOSIDES

Hellmut Bredereck, Leipzig, Germany; vested in the Alien Property Custodian

No Drawing. Application filed February 20, 1939

This invention relates to a process for the manufacture of nucleosides from nucleic acids. The invention is more particularly concerned with a process in which the nucleosides in nucleic acids are obtained by the action of bases on nucleic acids at an elevated temperature.

Attempts have already been made to isolate nucleasides from nucleic acids by means of ammonia at temperatures of 175–180°C in an autoclave, but the yields of the individual nucleosides which were obtained in this manner were only small. In particular the working up of the solution obtained by this process was an extremely laborious and complicated operation. Attempts to split up nucleic acids by means of alkali at temperatures below 175–180°C have hitherto always led to the production of nucleotides and not to the production of nucleosides. The production of nucleosides in a satisfactory manner by a chemical process has therefore not yet been accomplished.

On the other hand it is also known to obtain nucleosides from nucleic acids with relatively good yields by a fermentative hydrolysis. The disadvantage of a process of this nature lies mainly in the difficulty of producing a ferment suitable for the splitting up of the nucleic acids. Further, after the nucleic acid has been split up the ferment employed must again be removed from the solution by heating.

One object of the present invention is to obtain nucleosides from nucleic acids without it being necessary to make a special ferment and to effect the splitting up of the nucleic acid by purely chemical methods. A further object of the invention is to considerably shorten the time necessary for the hydrolysis of the nucleic acids as compared with the time necessary when the hydrolysis is effected with ferments. A still further object of the invention is to make it unnecessary to heat up the solution after the hydrolysis is complete for the purpose of removing the ferment.

As compared with the previously known purely chemical process of splitting up nucleic acids an object of the present invention is to considerably improve the yield of nucleosides and in particular to obtain approximately quantitative yields of nucleosides such as guanosin, adenisine, cytidine and uridine. Another object is to avoid, during the hydrolysis, any injury to the sensitive chemical compounds with which the present invention is concerned. In this connection a particular object of the invention is to obtain the nucleosides in a practically pure state and preferably directly in a crystalline form.

A still further object of the invention is to reduce the temperature necessary for the chemical hydrolysis of the nucleic acids so that the use of an autoclave can, in general, be dispensed with. Finally, yet another object of the invention is to simplify and cheapen the process of obtaining nucleosides from nucleic acids by recovering from the solution which is treated by the process of the invention the base which is employed for splitting up the nucleic acid and using it again for splitting up the next batch of fresh nucleic acid.

These and other objects of the invention are realised by splitting up nucleic acids in an alkaline medium at a temperature lying below 175–180°C and continuing the hydrolysis for a longer period than in the known processes which have only led to the separation of nucleotides. I have found that, by working in this manner, nucleic acids split up without trouble into nucleosides. Thus, for example, if nucleic acid is boiled under a reflux cooler in an alkaline solution, that is to say for example with an addition of caustic soda or potash solution or baryta water, or if the solution is merely heated on the water bath, the nucleic acid is split up with the formation of nucleosides. By working up the solution obtained in this manner I have succeeded in obtaining the nucleosides. It is true that the separation of the nucleosides from the solution obtained presents some difficulty because alkali phosphate is produced during the hydrolysis which sometimes prevents the crystallisation of the guanosine in the solution from proceeding smoothly.

For this reason therefore I prefer a method of operation in which organic bases are employed as the agent for splitting up the nucleic acids. The use of volatile organic bases such as pyridine, quinoline, aniline and their homologues, analogues and derivatives has proved to be particularly advantageous. Instead of such organic bases I can also use aliphatic bases such as, for example, mono-methylamine, diethylamine, trimethylamine and their homologues or derivatives. Thus, for example if nucleic acids are boiled for some time in aqueous pyridine under a reflux cooler it is readily split up to form nucleosides. The duration of the splitting-up process depends mainly on the degree to which the phosphoric acid is split off from the nucleic acid. This splitting off of phosphoric acid can be easily determined analytically by methods which are known

to the chemist. As distinguished from alkalies, pyridine as well as other volatile bases can be separated by distillation. In this way the guanosine can be directly isolated in crystalline form. The filtrate from the guanosine can be treated directly with picric acid whereby adenosine picrate is obtained in a really pure state. The decomposition of the adenosine picrate and the recovery of the pure adenosine proceeds without trouble. For this purpose the process described in my prior German specification No. 650,847, is preferably employed. Adinosine can however be obtained directly as such in crystalline form from the filtrate from the guanosine after removing the phosphoric acid and any impurities by methods which are known to the chemist.

As will be clear from what has already been stated above, the new process is carried out at considerably lower temperatures and without the use of an autoclave. By this means destruction of the sensitive chemical compounds which are in question, and, in particular, the nucleic acids and the nucleosides is avoided. The yields obtained are consequently decidedly improved and guanosine and adenosine in particular are obtained with nearly quantitative yields. When an organic base is used as the agent for splitting up the nucleic acid the working up of the solution is enormously facilitated, since when the splitting up is effected, as described in the technical literature by means of ammonia, a gelatinous mass is obtained which must first be purified. Further, in the latter case the ammonium phosphate formed must be removed, otherwise when picric acid is added it would precipitate together with the adenosine picrate in the form of the difficultly soluble ammonium picrate.

As compared with the fermentative splitting up of the nucleic acids, the present invention not only has the advantage that the process is independent of the ferment and its manufacture but the duration of the hydrolysis is considerably shortened, the yields are also improved and a product of greater purity is obtained. In particular the adenosine picrate obtained by the process of the present invention is in general purer than the picrate obtained when the splitting up is performed by fermentation.

The volatile base which is recovered by distillation from the solution produced by the process can be used again for the next batch of nucleic acid to be treated whereby the process is considerably simplified and the cost reduced. Owing to the adenosine being obtained in a pure crystallised state the process of obtaining the cytidine and uridine is improved and simplified.

The process will now be explained with the aid of the following example. The invention is in no way limited to the individual measures described in the example or to the exact sequence of operations as numerous modifications can be made which will be at once obvious to the technician.

Example

50 grams of yeast nucleic acid are boiled in 300 cc of aqueous pyridine under a reflux cooler until the phosphoric acid has been completely split off, that is to say for about 96 hours. After clarification with animal charcoal the solution is evaporated in vacuo. The residue is taken up in water and again evaporated. The guanosine which is insoluble after the addition of water is filtered off after cooling down in the ice chest. The yield is about 10 grams. The filtrate is treated with some NaOH for removal of the remains of pyridine and evaporated. The residue is taken up with water, the aqueous solution is heated slightly and treated with 25 grams of picric acid. After cooling in the ice chest the liquid is filtered off from the adenosine picrate which separates. The yield is about 18–22 grams. The pyrimidine nucleosides, cytidine and uridine, are obtained in known manner by working up the filtrate. To obtain the free adenosine directly, the filtrate from the guanosine is freed from phosphoric acid with baryta water and then from any impurities with lead acetate or with an organic solvent miscible with water. The adenosine crystallises out of the filtrate, which if necessary may be reduced. The filtrate can be worked up directly to cytidine and uridine.

HELLMUT BREDERECK.