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# METHOD OF PRODUCING NUCLEOSIDES BY CHEMICAL PROCESSING

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This application is a continuation-in-part of our application Serial No. 139,782, filed January 20, 1950, now U. S. Patent 2,698,844.

According to the already known method, the production of nucleosides by hydrolytic cleaving of nucleic acid can be carried through by a 3–4 hours' treatment with ammonia at a heat of 175–180° C. In this case a mixture of the purine- and pyrimidine-nucleosides is obtained.

The disadvantage of this process consists in realizing a bad yield and in being forced to work with pressure-vessels. If treated under more moderate conditions, e. g. at 115° C., the nucleic acid is only degraded into the mono-nucleotides.

For this reason it was suggested (German Letters Patent No. 693,416) to use volatile organic bases, e. g. pyridine, chinoline etc., a procedure by which the cleaving of nucleic acid will be accomplished in approximately 96 hours. The yield of guanosine and adenosine is considerably higher than that realized by using ammonia; however, pyridine is a very expensive cleaving-agent.

It is further known to carry through the cleavage of nucleic acids in an alkaline medium by adding of alkaline earth bases. This procedure has several disadvantages. In this way the adenosine formed by the cleavage of nucleic acid never can be obtained directly.

It was found as a surprise that the hydrolysis of the nucleic acid can also be done without employing the usual inorganic and organic bases if using a neutral aqueous solution at a pH value of 6.5–8, preferably 7–7.5, this pH value being kept constant by buffering in order to re-rendering inactive the phosphoric acid nascent at the cleaving-process. For buffering all buffering compounds may be used that are capable to maintain the mentioned pH-value. As well known, buffering compounds are materials, which in an aqueous solution at a given concentration show a determined pH-value and tend to maintain its pH-value even then if acid or basic material is added.

Inorganic or organic buffering compounds may be employed. The phosphoric acid, formed by the hydrolytic cleavage of nucleic acid is buffered and consequently prevented from a further cleavage of the nucleosides formed. Therefore the desired hydrolytic cleaving of the nucleic acid is not specifically due to the cleaving-agent but to means that maintain a determined range of pH.

If pressure-vessels are avoided the hydrolysis can often be carried through considerably faster than it was feasible up to now. Often the solubility-conditions are so favorable that at the cooling-down certain nucleosides as, for instance, guanosine precipitate immediately from the solution and may be obtained in a comparatively pure form. Thus, the nucleoside-yields being equal, this new method constitutes a considerable technical simplification and an essential lowering of costs, as compared with the present method.



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

*Example 1*

110 g. of unrefined nucleic acid are brought to suspension in 200 ml. of water, its pH being adjusted to 7 with soda-lye, and are diluted with 10–20 times the quantity of buffer-solution favoring a pH-value of 7 (sodium citrate and soda-lye) which is followed by 9–10 days boiling at the reflux-cooler. Then the guanosine precipitate is filtered off. Yield: 10 g. Hereafter the adenosine is separated directly or as a compound of difficult solubility.

*Example 2*

100 g. of unrefined nucleic acid with 70–75% pure nucleic acid content are brought to suspension in 200 ml. water, its pH being adjusted to 7.0 with soda-lye, and diluted with 1–2 liters of a buffering molar alkaline phosphate solution according to Sörensen, containing 70 parts of secondary alkaline phosphate and 30 parts of primary alkaline phosphate. By 7–10 days' boiling at the reflex-cooler the nucleic acid is cleaved. Then the guanosine precipitate is separated and the adenosine directly isolated from the hydrolytic solution or as a compound of difficult solubility. Yields: 12.2 g. guanosine and 10.5 g. adenosine.

*Example 3*

100 g. unrefined nucleic acid with 70–75% pure nucleic acid content are brought to suspension in 200 ml. water, its pH being adjusted to 7.0 with soda-lye, and diluted with 1–2 liters of a buffering molar alkaline phosphate solution according to Michaelis, containing 1 part primary alkaline phosphate and 2 parts secondary alkaline phosphate. By 7–10 days' boiling at the reflux-cooler the nucleic acid is cleaved. Then the guanosine precipitate is separated and the adenosine directly isolated from the hydrolytic solution or as a compound of difficult solubility. Yields: 12.5 g. guanosine and 10.3 g. adenosine.

*Example 4*

100 g. of unrefined nucleic acid with 70–75% pure nucleic acid content are brought to suspension in 200 ml. water, its pH being adjusted to 7.0 with soda-lye, and diluted with 1–2 liters of a buffering molar alkaline phosphate solution according to Clark and Lubs, consisting of 296 ml. normal solution of sodium hydrate and 500 ml. normal solution of primary alkaline phosphate. By 7–10 days' boiling at the reflux-cooler the nucleic acid is cleaved. Then the guanosine precipitate is separated and the adenosine directly isolated from the hydrolytic solution or as a compound of difficult solubility. Yields: 11.5 g. guanosine and 10.0 g. adenosine.

*Example 5*

100 g. of unrefined nucleic acid with 70–75% pure nucleic acid content are brought to suspension in 200 ml. water, its pH being adjusted to 7.0 with soda-lye, and diluted with 1–2 liters of a buffering molar alkaline phosphate solution according to Kolthoff, containing 623 parts primary calcium phosphate and 377 parts borax. By 7–10 days' boiling at the reflux-cooler the nucleic acid is cleaved. Then the guanosine precipitate is separated and the adenosine directly isolated from the hydrolytic solution or as a compound of difficult solubility. Yields: 11.6 g. guanosine and 10.2 g. adenosine.

*Example 6*

100 g. of unrefined nucleic acid with 70–75% pure nucleic acid content are brought to suspension in 200 ml. water, its pH being adjusted to 7.0 with soda-lye, and diluted with 1–2 liters of a buffering molar alkaline phosphate solution according to McIlvaine, consisting of 165 parts 4 times molar solution sodium phosphate and 35 parts double molar citric acid. By 7–10 days' boiling at the reflux-cooler the nucleic acid is cleaved. Then the guanosine precipitate is separated and the adenosine directly isolated from the hydrolytic solution or as a compound of difficult solubility. Yields: 10.5 g. guanosine and 8.2 g. adenosine.

*Example 7*

100 g. of unrefined nucleic acid with 70–75% pure nucleic acid content are brought to suspension in 200 ml. water, its pH being adjusted to 7.0 with soda-lye, and thereby are dissolved. Then 2.7 liters of a buffering veronal-acetate solution according to Michaelis are added, consisting of:

| | Ml. |
|---|---|
| 7 times molar sodium-acetate solution+7 times molar sodium diethylbarbiturate solution | 500 |
| 8.5% sodium chloride solution | 200 |
| 5 times molar hydrochloric acid | 600 |
| Water | 1400 |

By 7–10 days' boiling at the reflux-cooler the nucleic acid is cleaved. Then the guanosine precipitate is separated and the adenosine directly isolated from the hydrolytic solution or as a compound of difficult solubility. Yields: 10.4 g. guanosine and 8.5 g. adenosine.

The invention may be further modified and embodied within the scope of the subjoined claims.

We claim:

1. In a process for the production of nucleosides by hydrolytic cleavage of a nucleic acid by heating the nucleic acid in an aqueous solution the improvements consisting in adjusting the aqueous solution of the nucleic acid to a pH-value of about 6.5 to 7.5 and then heating the same in the presence of 10 to 20 times the amount by weight, calculated on nucleic acid, of a 1 to 7 times molar buffering compound, and herewith maintaining the pH-value at 6.5 to 7.5 by buffering the phosphoric acid split off during hydrolysis.

2. In a process for the production of nucleosides according to claim 1, in which the buffering compound is inorganic.

3. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound consists of a molar solution containing 70 parts secondary alkaline phosphate and 30 parts primary alkaline phosphate.



4. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound consists of one part primary alkaline phosphate and two parts secondary alkaline phosphate in a molar solution.

5. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound consists of 296 ml. normal sodium hydrate and 500 ml. normal primary alkaline phosphate.

6. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound consists of 623 parts primary calcium phosphate and 377 parts borax.

7. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound consists of 165 parts 4 times molar sodium phosphate and 35 parts double molar citric acid.

8. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound is a solution containing sodium citrate and soda-lye.

9. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound is a solution consisting of:

| | Ml. |
|---|---|
| 7 times molar sodium-acetate solution+7 times molar sodium diethylbarbiturate solution | 500 |
| 8.5% sodium chloride solution | 200 |
| 5 times molar hydrochloric acid | 600 |
| Water | 1400 |

10. In a process for the production of nucleosides according to claim 1, in which the buffering compound is organic.

11. In a process for the production of nucleosides according to claim 1, in which the molar buffering compound is selected from the group of organic and inorganic compounds.

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