

10 OCT. 1962

PATENT SPECIFICATION

NO DRAWINGS

827,441

Date of Application and filing Complete Specification Jan. 23, 1958.

No. 2305/58.

Application made in Czechoslovakia on Feb. 28, 1957.

Complete Specification Published Feb. 3, 1960.

Index at acceptance:—Class 2(3), C3A12(A4A:A4B:B2:C6).

International Classification:—C12d.

COMPLETE SPECIFICATION**A method of preparing Ribosides of Nucleic Acid Bases**

We, SPOFA, SPOJENE FARMACEUTICKE ZAVODY, NARODNI PODNIK, a Czechoslovakian corporation of No. 11a, Husinecka, Prague XI, Czechoslovakia, 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:—

This invention relates to a method of preparing ribosides of nucleic acid bases.

Ribosides of nucleic acid bases, such as riboside of 6-azauracil and of its 6-substituted derivatives possess valuable cancerostatic and bacteriostatic properties. Some of them also show antileukemic activity.

The present invention has for its object a biochemical synthesis of ribosides of nucleic acid bases. According to the invention, the base of a nucleic acid or a derivative of such base, particularly 6-azauracil, is exposed to a fermenting saccharide-containing culture of *Escherichia coli* and the product is isolated by adsorption on charcoal or on another adsorptive material.

The process may be easily carried out in the usual equipment of the fermentation industry. The process is very simple and may be analytically controlled without difficulties.

The yields, computed on the base of nucleic acid, are almost quantitative.

EXAMPLE.

300 litres of a cultivating medium are prepared in the following manner: 1080 grs. of glucose are dissolved in 5 litres of water and the sterilized solution is added under aseptic conditions to the main part of an aqueous solution, containing:—

	Grams
Ammonium sulphate - - -	792
Sodium chloride - - -	174
Magnesium sulphate (cryst.) -	72
Calcium chloride (cryst.) -	3
Ferrous sulphate (cryst.) -	4.5
Dihydrogen potassium orthophosphate - - -	816
Disodium hydrogen orthophosphate - - -	8595

This medium is inoculated with one per cent of inoculum, consisting of a 24 hours old culture of *Escherichia coli* in the same cultivating medium. After a cultivation lasting 6 hours a sterile solution of 6-azauracil is added in such an amount as to obtain the concentration of 7.10^{-4} M. The fermentation is then continued for a further 5—15 hours at 37° C. without agitation and aeration. The medium is then cooled down with water and the 6-azauracil riboside is adsorbed on charcoal in a column and eluted with an aqueous 50 per cent ethanol containing one per cent of ammonia. The eluate is evaporated and the pure product is obtained by recrystallization from butanol saturated with water or chromatographically by means of a cellulose column. The yield of 6-azauracil riboside is over 90 per cent, computed on 6-azauracil. The pure substance melts at 160—161° C.

WHAT WE CLAIM IS:—

1. A method of preparing ribosides of nucleic acid bases, particularly 6-azauracil riboside, comprising exposing a base of a nucleic acid to a fermenting-saccharide-containing culture of *Escherichia coli* and isolating the product from the clear medium by adsorption on an adsorptive material.

[Price 3s. 6d.]

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[Price 3s. 6d.]



2. A method of preparing ribosides of nucleic acid bases according to the example.
3. Ribosides of nucleic acid bases when prepared by the method of Claim 1.
- 5 4. Ribosides when prepared by the method of Claim 2 or its obvious chemical equivalents.

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