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IODINATION OF THE HETEROCYCLIC BASES IN NUCLEOSIDES, NUCLEOTIDES, NUCLEOSIDE-5'-POLYPHOSPHATES, AND NUCLEIC ACIDS

David Lipkin, St. Louis, Mo., assignor to the United States of America as represented by the Secretary of the Department of Health, Education, and Welfare
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This invention relates to the halogenation of the heterocyclic base in nucleosides, nucleotides, nucleoside-5'-polyphosphates and nucleic acids. It aims generally to provide a process or method by which direct halogenation, e.g. iodination, of the bases of such sensitive molecular structures can be effected, to afford halogenated materials of therapeutic and general biochemical interest and which can serve as new and useful materials for biochemical investigations.

Objects of the invention, severally and interdependently, are to provide methods for the direct halogenation of the materials concerned; to provide such methods which are rapid; to provide such methods which can take place at or below room temperature; to provide such methods which can be carried out in media which are neither strongly acidic or basic; to provide such methods in which desirable yields can be obtained; and to provide new compounds made available by the new procedures.

The invention resides in the new and useful process steps and combinations and in the new compounds, herein disclosed, and is more fully defined in the appended claims.

To facilitate an understanding of the invention a general description thereof will first be presented followed by illustrative examples of the invention.

GENERAL DESCRIPTION

Known processes for the iodination of the less sensitive pyrimidine nucleosides or nucleotides make use of iodine in aqueous sodium hydroxide or a refluxing mixture of iodine and nitric acid. Such procedures have not been, and so far as is known cannot be, used for the direct iodination of nucleoside-5'-polyphosphates and polynucleotides, and no procedure has been found described in the literature which is applicable to the direct iodination of the more sensitive purine derivative nucleosides, e.g. adenosine, guanosine, deoxyguanosine, or the corresponding nucleotides. By the present invention nucleoside polyphosphate, more particularly uridine-5'-triphosphate, are converted directly to the 5-iodo compounds in good yield, and processes are made available which are applicable for direct iodination of the sensitive purine derived nucleosides and nucleotides as well as for those derived from pyrimidines.

For ease of reference, the heterocyclic base moiety of the nucleosides or nucleotides is herein sometimes called the purine body moiety, when a derivative of purine (e.g. when a moiety of xanthine, adenine, guanine, deoxyguanine, etc.) and sometimes called the pyrimidine body moiety when a derivative of pyrimidine (e.g. when a moiety of uridine, thymine, cytidine, deoxyuridine, etc.). Also except when otherwise specifically stated, the combination of the base moiety with the sugar moiety of course is termed nucleoside, and the further combination of the nucleoside moiety with one or more phosphoric acid moieties is termed a nucleotide whether or not such phosphoric acid moiety or moieties are present in the acid or salt form. Similarly the nucleosides, nucleotides, etc. from a particular purine body may be sub-termed compounds of that body, e.g. guanine compounds, adenine compounds, xanthine compounds; and those from a particular pyrimidine body may be sub-termed compounds of such body, e.g. uracil compounds, thymine compounds, cytosine compounds.

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Iodinated nucleosides and nucleotides are materials and intermediates of chemotherapeutic and/or biochemical interest and utility. Their incorporation into nucleic acids increases the sensitivity of the nucleic acids to radiation. Also 5'-iododeoxyuridine has been reported to be useful for other purposes, and iodouracil derivatives have given indication of being useful for the control of some virus infections. Thus the present invention improves the availability of known iodinated compounds as well as providing new iodinated compounds from sensitive precursor materials.

In accordance with one embodiment of the present invention, applicable particularly to the more sensitive purine-body nuclei, direct iodination of the heterocyclic nuclei in nucleosides, nucleotides, nucleoside-polyphosphates, and polynucleotides is effected with the aid of N-iodo-succinimide, in a sulfoxide solvent, e.g. dimethyl sulfoxide, and preferably in the presence of a sulfur catalyst selected from the group consisting of elemental sulfur and the alkyl, aryl and heterocyclic di-sulfides, especially n-butyl-di-sulfide (C_4H_9S)₂.

In accordance with another embodiment of the invention, applicable particularly to the pyrimidine body nuclei, direct iodination of the heterocyclic nuclei in nucleosides, nucleotides, nucleotide-polyphosphates, and polynucleotides is effected with the aid of a mixed halogen compound as iodinating agent in a sulfoxide or carboxylic acid amide solvent, preferably in the presence of a sulfur catalyst as above defined when sulfoxide solvent is employed, and preferably in the presence of 2-t-butyl-pyridine when mixed halogen reagent is employed. The mixed halogens include iodine monochloride, which is the preferred one of these reagents, and also iodine, iodine monobromide, iodine trichloride, iodine pentafluoride and cyanogen iodide.

With these reagents used as disclosed above and in the following examples, compounds have been prepared on a preparative scale by direct iodination of:

uridine
uridine-3'-phosphoric acid
uridine-5'-phosphoric acid (UMP)
uridine-5'-tri-phosphoric acid (UTP)
guanosine
guanosine-2'(3')-phosphoric acid, and
xanthosine.

By like procedures, compounds have been prepared on a micro-scale from the nucleosides corresponding to:

deoxyguanosine
adenosine, and
cytidine.

As above noted, two types of compounds, carboxylic acid amides and sulfoxides, are of interest as media in which to carry out the iodinations. Specifically, N-ethylacetamide and dimethyl sulfoxide were found to be most useful. N-ethylacetamide is the solvent of choice if iodine monochloride is being used as the iodinating agent, since the reaction is more rapid and complete than in dimethyl sulfoxide. In fact, the iodination of uridine by means of iodine monochloride appears to be inhibited by dimethyl sulfoxide. On the other hand, N-ethylacetamide is completely unsatisfactory as a solvent in which to carry out iodinations using N-iodosuccinimide as the reagent.

When iodine monochloride is used as the iodinating agent, one mole of hydrogen chloride is formed per mole of iodine monochloride used. This is disadvantageous in the iodination of acid-sensitive substances such as nucleoside-5'-polyphosphates and nucleic acids. Fortunately, it was found that iodine monochloride may be used as an iodinating agent in the presence of 2-t-butylpyridine. This sterically hindered base does not prevent

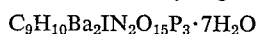
iodination and in some cases actually acts as a mild catalyst. On the contrary, other pyridine bases (pyridine, 2-picoline, 2-ethylpyridine, 2-isopropylpyridine and 2, 6-lutidine) markedly inhibit the iodination of uridine.

Iodination of adenosine, guanosine or uridine by means of N-iodosuccinimide at room temperature, in pure dimethyl sulfoxide as solvent, is an extremely slow reaction and the yields of iodinated nucleoside are very poor. It was found, however, that this reaction is subject to marked catalysis using sulfur catalysts. For example, by the use of N-iodosuccinimide in dimethyl sulfoxide uridine can be quantitatively iodinated in one hour at room temperature by the addition of n-butyl disulfide in low concentrations to the reaction mixture. Other alkyl, aryl and heterocyclic disulfides are effective catalysts also. In fact, even elementary sulfur is a moderately effective catalyst. The monosulfides, i.e., n-butyl sulfide, are quite ineffective catalysts and this is true also for a number of other substances such as selenium and organic selenides.

The advantageous features and new and useful aspects of the invention, some of which are described generally above, will be readily appreciated by those skilled in the art from the following examples, which are illustrated but not restricted of the invention:

Example 1

To a solution of the tetraethylammonium salt of UTP (0.141 mmole) and 5 μ l. (0.026 mmole) of n-butyl disulfide in 2.0 ml. of anhydrous dimethyl sulfoxide was added over a 1-hour period, with continuous stirring, a solution of 130 mg. (0.58 mmole) of N-iodosuccinimide in 0.5 ml. of sulfoxide. The UTP contained approximately 6% UDP and 0.6% P_i. After an additional 2 hours the reaction mixture was poured into a solution of 267 mg. (0.63 mmole) of barium iodide in 20 ml. of methanol. This mixture was kept at -20° overnight, and the subsequent operations then were carried out at a temperature of 0-5°. The solid was recovered by centrifugation and washed three times with 20-ml. volumes of methanol. The solid was dissolved in about 5 ml. of 0.1 N hydrobromic acid, and then the pH of the resulting solution was increased by the addition of 1 N barium hydroxide. The solid that precipitated between pH 3.1 and 5.5 was recovered by centrifugation. The product (116 mg.) was washed with methanol and ether and finally air-dried. Elemental analysis gave



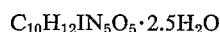
Calculated: I, 12.61; N, 2.78; total P, 9.23, P_i, 0.00, 7-minute hydrolyzable P, 6.15%. Found: I, 12.35; N, 2.44; total P, 9.28, P_i, 0.87, 7-minute hydrolyzable P, 5.87%.

Column chromatography on Dowex 1-X2 (formate) showed that 90% of the product was 5-iodo-UTP. The principal ultraviolet-absorbing impurities were UTP, UDP (uridine di-phosphate), and presumably 5-iodo-UDP. At pH 2.2, $\lambda_{max}=213$ m μ ($\epsilon=10,600$) and 284 m μ ($\epsilon=6,360$) and $\lambda_{min}=245$ m μ ($\epsilon=2,740$); at pH 7.0, $\lambda_{max}=213$ m μ ($\epsilon=10,700$) and 284 m μ ($\epsilon=6,260$) and $\lambda_{min}=246$ m μ ($\epsilon=2,720$); at pH 12, $\lambda_{max}=273$ m μ ($\epsilon=5,290$) and $\lambda_{min}=251$ m μ ($\epsilon=4,040$). Paper chromatography (descending, Whatman 3MM paper) in the developing solvent 5% disodium hydrogen phosphate saturated with isoamyl alcohol gave: R_F of UTP, 0.89; R_F of 5-iodo-UTP, 0.94. Paper electrophoretic mobilities, relative to UMP, were: in 0.05 M citrate buffer (pH 5.2) M_r of UTP, 1.85, and M_r of 5-iodo-UTP, 1.72; in 0.05 M borate buffer (pH 9.2), M_r of UTP, 0.87, and M_r of 5-iodo-UTP, 0.93. 5-iodo-UTP was degraded by means of 60% hydrofluoric acid to 5-iodo-UMP (-50°, 1 hour) and 5-iodouridine (0°, 1 hour).

Example 2

A solution of 1.0 g. (3.5 mmoles) of guanosine, 7.8 g. (35 mmoles) of N-iodosuccinimide, and 50 μ l. (0.26 mmole) of n-butyl disulfide in 10 ml. of dimethyl sul-

foxide was allowed to stand at room temperature for 17 hours. The crude reaction product was precipitated by adding 200 ml. of cold acetone to the reaction mixture and allowing it to stand at -20° for several hours. It was purified further by reprecipitation in the same manner. This solid (1.2 g.) was recrystallized from hot water and the resulting white needle-like crystals were air-dried (1.0 g., 70% yield).



Calculated: C, 26.43; H, 3.74; I, 27.97; N, 15.41%. Found: C, 26.66; H, 3.68; I, 27.61; N, 15.24%.

Iodoguanosine has $[\alpha]_D -14.2^\circ$ (c. 2, in dimethyl sulfoxide); for guanosine itself, $[\alpha]_D$ is -30.8° (c. 2, in dimethyl sulfoxide). At pH 2, $\lambda_{max}=260$ m μ ($\epsilon=17,900$) and $\lambda_{min}=228$ m μ ($\epsilon=3,750$); at pH 7, $\lambda_{max}=262$ m μ ($\epsilon=18,300$) and $\lambda_{min}=228$ m μ ($\epsilon=3,750$); at pH 12, $\lambda_{max}=270$ m μ ($\epsilon=14,800$) and $\lambda_{min}=236$ m μ ($\epsilon=4,200$). Iodoguanosine, relative to guanosine, has an R_F of 2.43 (1-butanol-water-formic acid, 76:10:14 by volume) and an M_r (electrophoresis, 0.05 M borate buffer, pH 9.2) of 0.93. It is quantitatively converted by means of nitrous acid to a compound that is identical with a sample of iodoxanthosine prepared by the direct iodination of xanthosine. Iodoguanosine, furthermore, is degraded by means of 60% hydrofluoric acid (approximately 25°, 1 hour) to ribose and a heterocyclic base that exhibits chromatographic, electrophoretic, and spectral properties to be expected of iodoguanine. The data are in agreement with the conclusion that the nucleoside is 8-iodoguanosine. Guanosine is not iodinated by means of ICl.

Example 3

Cytidine (10 mg., 0.041 mmole) was dissolved in 0.3 ml. of anhydrous N-ethylacetamide, and to this solution were added 40 μ l. of 2 M ICl in carbon tetrachloride (0.080 mmole). The reaction mixture was allowed to stand at room temperature for 1 hour. It then was diluted with water and an aliquot was streaked on paper. After chromatography in 1-butanol-acetic acid-water (4:1:1 by volume), only one ultraviolet-absorbing band was found; R_F 0.28. Cytidine in this solvent has an R_F of 0.18. Relative to cytidine, the new substance has an electrophoretic mobility on paper in 0.05 M borate buffer, pH 9.2, of 0.89. The new compound, whose behavior is consistent with its being 5-iodocytidine, in 0.1 N HCl has $\lambda_{max}=308$ m μ and $\lambda_{min}=261$ m μ . 5-iodo-2'-deoxycytidine is reported to have $\lambda_{max}=308$ to 309 m μ at pH 2. Based on the extinction coefficient reported for this deoxy compound, the yield of 5-iodocytidine was 85%.

Example 4

Example 3 was repeated, there being included in the solution a calculated quantity of 2-t-butylpyridine sufficient to neutralize the hydrogen chloride as formed. It was found that 2-t-butylpyridine does not inhibit iodination by means of ICl, although other pyridine bases, i.e. pyridine, 2-isopropylpyridine, and 2,6-lutidine, do.

Example 5

Repetition of the procedure of Example 4 using uridine instead of cytidine also rapidly produced iodo-uridine as identified in Example 1.

Example 6

Repetition of Example 5 but with dimethyl sulfoxide as solvent also rapidly produced iodo-uridine as identified in Example 1.

Example 7

Repetition of Example 1 but with uridine-3'-phosphoric acid in place of the UTP rapidly produced a good yield of the corresponding iodinated product.

Example 8

Repetition of Example 1 but with uridine-5'-phosphoric acid in place of the UTP rapidly produced a good yield of the corresponding iodinated product.

Example 9

Repetition of Example 2 but with guanosine-2'(3')-phosphoric acid in place of the guanosine, produced a good yield of the corresponding iodinated product.

Example 10

Repetition of Example 2 but with deoxyguanosine in place of the guanosine, produced a similar yield of the corresponding iodinated product.

While there have been described herein what are at present considered preferred embodiments of the invention, it will be obvious to those skilled in the art that minor modifications and changes may be made without departing from the essence of the invention. It is therefore to be understood that the exemplary embodiments are illustrative and not restrictive of the invention, the scope of which is defined in the appended claims, and that all modifications that come within the meaning and range of the equivalency of the claims are intended to be included therein.

The invention described herein is assigned to the Government of the United States and it is prayed that the present application be accorded fee exempt status in accordance with the opinion of the Comptroller General No. B-111,648 (96 USPQ 453; 98 USPQ 238 and 6607.01 MPEP).

A paper presenting details of the present invention, and of the characterization of the iodinated products obtained has been published by and with the consent of the applicant during the year next preceding the filing of the present application in the Journal of Biological Chemistry, vol. 238, No. 6 (published June 25, 1963) at pages PC 2249-2251, which paper is incorporated herein by reference.

I claims:

1. Process for the iodination of the heterocyclic bases in sensitive derivatives thereof selected from the class consisting of nucleotides, nucleosides, nucleoside-polyphosphates and nucleic acids, which comprises

- (a) reacting the sensitive derivative with N-iodo-succinimide
- (b) in a sulfoxide solvent
- (c) in the presence of a sulfur catalyst selected from the group consisting of elemental sulfur and the alkyl, aryl, and heterocyclic di-sulfides, and
- (d) recovering the iodinated product.

2. Process according to claim 1, wherein the solvent is dimethylsulfoxide.

3. Process according to claim 1, wherein the catalyst is n-butyl-disulfide.

4. Process according to claim 1, wherein the material iodinated is UTP.

5. Process according to claim 1, wherein the material iodinated is a uracil compound,

6. Process according to claim 1, wherein the material iodinated is a guanine compound.

7. Process according to claim 1, wherein the material iodinated is a deoxypentose compound.

8. Process according to claim 1, wherein the material iodinated is an adenine compound.

9. Process according to claim 1, wherein the material iodinated is a xanthine compound.

10. Process for the iodination of the heterocyclic bases in sensitive derivatives thereof selected from the class consisting of nucleotides, nucleosides, nucleoside-polyphosphates and nucleic acids, which comprises

- (a) reacting the sensitive derivative with iodine monochloride
- (b) in a carboxylic acid amide solvent and
- (c) recovering the iodinated products.

11. Process according to claim 10, wherein the reaction is carried out in the presence of 2-t-butyl pyridine.

12. Process according to claim 10, wherein the solvent is N-ethyl acetamide.

13. Process according to claim 10, wherein the material iodinated is a uracil compound.

14. Process according to claim 10, wherein the material iodinated is a cytosine compound.

15. Process for the iodination of the heterocyclic bases in sensitive derivatives thereof selected from the class consisting of nucleotides, nucleosides, nucleoside-polyphosphates, and nucleic acids, which comprises

- (a) reacting the sensitive derivative with iodine monochloride
- (b) in a sulfoxide solvent, and
- (c) recovering the iodinated products, said sensitive derivative being a uracil compound.

16. A compound selected from the group consisting of 8-iodo-guanosine, its nucleotides and nucleic acids.

17. A compound selected from the group consisting of 8-iodo-deoxyguanosine, its nucleotides and nucleic acids.

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LEWIS GOTTS, *Primary Examiner.*

J. R. BROWN, *Assistant Examiner.*