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Preparation of Furoic Acid from Ribo Nucleic Acid

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Methods have recently been worked out in this laboratory for the preparation of the nitrogenous components of ribo nucleic acid (PNA) with a view to applying them to tracer studies [1–4]. Ribose, the carbohydrate of PNA has, however, not so far been included in the isotopic investigations. The main reason for this has been the lack of a suitable method for the preparation of this compound from small amounts of the PNA. The purpose of this paper is to present such a method and at the same time to describe its application to a problem, where glycine marked in the carboxyl group with C^{14} has been used as a precursor for the polynucleotides.

It seemed that the crystallisation of ribose from amounts of as little as utmost 200 mg of PNA would prove very difficult. Because of this it was decided to transform the ribose into furoic acid (pyromuic acid), a compound which is relatively easy to characterize and to obtain in pure form. This was achieved by preparing furfural from the ribose containing material by steam distillation in the presence of strong mineral acid, oxidation of the furfural to furoic acid with ammoniacal silver oxide, and purification of the acid by sublimation *in vacuo*.

EXPERIMENTAL

Preparation of PNA. The preparation of the polynucleotides and then separation into desoxyribo nucleic acid and PNA was carried out according to Hammarsten [1].

Preparation of furfural. Furfural was first prepared from the PNA. As usually occurs when furfural is prepared from PNA the aldehyde is formed mainly from the ribose bound to the purines and not from that bound to the pyrimidines. For the conversion of pentoses to furfural different authors have used distillation with anhydrous zinc chloride, trichloro acetic acid, phosphoric acid and strong mineral acids [5–7]. The best yields have

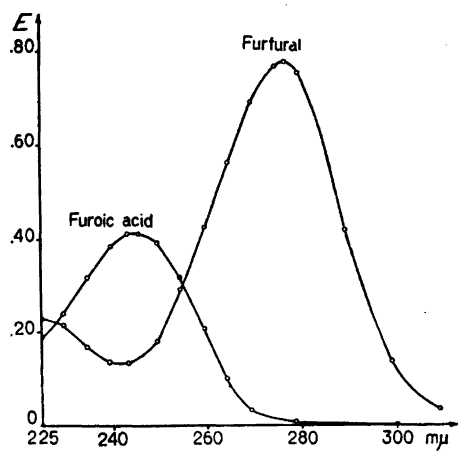

Fig. 1. Absorption curve in ultraviolet of water solutions of furfural and furoic acid.

usually been obtained with sulphuric and hydrochloric acids. Of the two hydrochloric acid gives a somewhat better yield (about 20 % more than does sulphuric acid). It is, however, very important for the oxidation of the furfural that the furfural solution after distillation should not contain any chloride ions. This could never be achieved when hydrochloric acid was used not even if the solution was twice redistilled with steam.

For this reason sulphuric acid was chosen for the preparation of furfural. 100—200 mg PNA were dissolved in 75 ml 9 N sulphuric acid. The solution was subjected to slow steam distillation for about 3 hours, the velocity of the distillation being about 30—40 ml per hour.

At the beginning of the investigation the distillation was considered finished, when a drop of the distillate gave no colour when applied to an anilin acetate paper [7]. Later the control of the distillation was carried out by taking advantage of the characteristic absorption in the ultra violet of the furan ring [5]. The absorption (Fig 1) was read in the Beckman photometer against water at the absorption maximum (277 mμ). For calculating the molar extinction coefficient a standard solution was prepared by twice redistilling *in vacuo* a preparation of furfural obtained from Eastman Kodak. This product had a specific gravity of 1.1581 at 20° and a boiling point of 159° C (1.1598 and 161.7° respectively for authentic material). The molar extinction coefficient was 14.800 at 277 mμ.

By following the absorption of the distillate it was possible to follow the course of the experiment (Table 1).

Oxidation of furfural. The formation of furoic acid from furfural has been carried out in various ways [9]. Only one method, however, that of oxidation

Table 1. Distillation of furfural from 250 mg PNA followed by means of lightabsorption in the ultraviolet.

Time minutes	E_{277}	Furfural obtained mg
0—30	111.5	18.1
30— 50	56.0	9.1
50— 70	15.5	2.5
70—100	26.8	4.3
100—150	6.8	1.0

of the pentose with silver oxide seemed likely to afford reasonable good yields on the mg scale. This method has even been used for quantitative estimation of furfural [10].

Slightly more than the amount of silver oxide theoretically necessary was freshly precipitated from a *N* solution of silver nitrate by the addition of 2 *N* sodium hydroxide. The precipitate was centrifuged off and washed with 2 × 5 ml of water. Afterwards it was dissolved in the smallest possible amount of 3 *N* ammonia and added to the furfural solution (about 100 ml). The oxidation was allowed to proceed for one minute at 100°. The solution was then evaporated to dryness in vacuo in order to remove all the ammonia. About 5 ml of hot water were added to the dry residue and the resulting suspension was centrifuged. The sediment was extracted with 2 × 5 ml hot water and the supernatants were combined. In order to decompose the silver furoic acid an excess of warm 0.1 *N* HCl was added. The silver chloride was centrifuged off and washed twice with hot water. The combined solutions were again evaporated to dryness in vacuo. The dry residue was extracted several times with ether, transferred to a small dish and the solution evaporated to dryness. In this way the furoic acid was collected on as small a surface as possible. The solid material was scraped of the walls of the dish and transferred with a small spatula to the sublimation apparatus (Fig. 2). The last traces of the furoic acid were transferred to the apparatus with the aid of a small amount of ether, which was evaporated before sublimation. This process which is described below was employed as the final stage of purification.

Furoic acid like furfural has a very characteristic absorption in the ultra violet [8]. The absorption curve (Fig. 1) and the molar extinction coefficient were determined on a synthetic specimen, which was prepared according to Wilson [11]. It had a melting point of 130—131° (132—133° for authentic material), an absorption maximum at 245 mμ and a molar extinction coefficient of

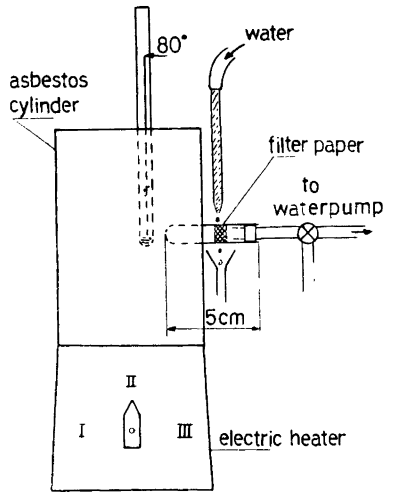

Fig. 2. Apparatus for microsublimation.

11.100. Furoic acid shows practically no absorption at 277 mμ which is the absorption maximum for furfural. Whereas at 245 mμ furoic acids has an absorption maximum and furfural a minimum (Fig. 1).

This makes it possible to follow the oxidation of furfural to furoic acid by measuring the ultra violet absorption of the solution during the reaction (Table 2).

Purification of furoic acid. A small test tube containing the crude product was immersed to half its height in an air bath of about 80°, and evacuated by a water pump. The upper part of the tube was surrounded by a strip of filter paper on which water was allowed to drip continuously. In this way the furoic

Table 2. The oxidation of furfural to Ag-furoic acid followed by measurement of absorption in the ultra violet. The quotient $\frac{E_{245}}{E_{260}}$ *served as measure of the course of the oxidation. The quotient of a standard of Ag-furoic acid was 2.13.*

Time	E_{245}	E_{260}	E_{277}	$\frac{E_{245}}{E_{260}}$
0	0.110	0.368	0.610	0.33
10 sec	0.147	0.325	0.570	0.45
20 »	0.410	0.245	0.110	1.57
40 »	0.460	0.225	0.025	2.04
1 min	0.450	0.210	0.015	2.14
3 »	0.460	0.220	0.012	2.09
4 hours	0.410	0.194	0.002	2.11

Table 3. The radioactivity per minute of about 15 mg $BaCO_3$ obtained from the various substances.

Material	Spec. activity, counts	Dilution of glycine carboxyl
Glycine administered	15 000	2
Mixed Polynucleotides	90	330
Furoic acid (ribose)	78	380
'Protein'	42	720

acid was sublimed on to a narrow area under the filter paper. The sublimation was allowed to proceed for 12 hours. At the end of this time the tube was cut and the acid collected. The melting point of the white and crystalline product varied from 129 to 131 degrees.

The over all yield of the whole procedure from PNA to furoic acid was 40—50 % calculated on the assumption of a tetranucleotide.

Experiments with $CH_2(NH_2) \cdot C^{14}OOH$. The present method has been tried on polynucleotides of chicken liver obtained during an experiment by Hammarsten *et al.*[12]. In this work one chicken received intraperitoneally a total of 3 g labelled glycine, divided into 4 doses at 6 hourly intervals. The animal was sacrified 12 hours after the last injection and the liver dried immediately with alcohol and ether.

The polynucleotides were prepared according to Hammarsten [1] and a 'protein' fraction was obtained by extraction of the dry organ powder with hot trichloro acetic acid as described by Schneider [13]. The ribose of the PNA was prepared as described above. The carbon from all samples was converted to $BaCO_3$ and the activity measured in a Geiger-Müller counter. The results are summarized in Table 3.

The higher level of C^{14} in PNA ribose compared with that in 'protein' indicates that there is a rather significant synthesis of PNA-ribose from glycine. On the other hand the isotope content of the mixed polynucleotides is somewhat higher than that of ribose. It seems best to postpone a more detailed discussion until other precursors for PNA-ribose have been investigated.

SUMMARY

A method for the isolation of furoic acid from small amounts of ribo nucleic acid is described.

This method has been used in an isotopic experiment, where glycine labelled with C^{14} in the COOH group has been used as precursor for ribo nucleic acid.

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