BRIEF COMMUNICATIONS

THE CHEMICAL STRUCTURE OF THE PRODUCTS OF THE TRANSFORMATION OF NATURAL PYRIMIDINE BASES AND 5-HALOURACILS BY MYCOBACTERIA

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It has been established that in the transformation of natural pyrimidine bases and 5-halouracils by strains of the genus Mycobacterium the following are formed as intermediates: from uracil, cytosine, and 5-iodouracil, barbituric acid; from thymine, 5-methylbarbituric aicd; and from 5-fluoro-, 5-chloro-, and 5-bromouracils, the corresponding 5-halobarbituric acids.

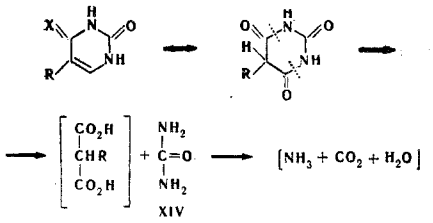
Compounds of the pyrimidine series are distributed widely in nature and play an important part in processes of vital activity. At the present time, a considerable amount of material has accumulated on the chemistry of the pyrimidine derivatives, but the conversions of the pyrimidines in biological systems have still been studied inadequately. Particularly convenient subjects for the performance of such investigations are microorganisms. The present paper gives the results of a study of the routes and intermediate products of the transformations of natural pyrimidine bases (uracil, thymine, and cytosine), and also of various 5-halouracils, by developing cultures of two strains of the genus Mycobacterium. These strains were isolated from the soil and, as we have established, can be grown successfully on synthetic media containing a pyrimidine derivative as the sole source of carbon and nitrogen or only of nitrogen.

In the experiments on the microbiological transformation of the natural pyrimidine bases (I–III), a synthetic medium of the following composition was used: 0.01% — KH_2PO_4, 0.003% — $MgSO_4 \cdot 7H_2O$, 0.001% — $FeCl_3$, 0.05–0.1% pyrimidine derivative, and distilled water to 100%. In the experiments on the transformation of the 5-halouracils (IV–VII), additional sources of carbon were added to the above synthetic medium, succinic acid (0.1%) and glycerol (0.4%), in combination, being used for this purpose. In all the experiments, a mixture of microelements containing the ions Mn^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}, MoO_4^{2-} and BO_3^{3-} was also added to the synthetic media, and the reaction of the initial medium was adjusted by sodium hydroxide solution to pH 7.2. In some experiments, discussed below, as well as the additional sources of carbon, additional sources of nitrogenous nutriment were also added to the medium. The medium was sterilized at 0.5 atm gauge for 30 min, and in the work with the 5-halouracils bacterial filters were used. As seed material we used 24-hour cultures of the strains mentioned above, grown on meat-peptone agar slants. The amount of meat material added was 5–10 mg per liter of medium. Incubation was carried out at 28° C with vigorous stirring and aeration (750-ml flasks, each containing 100 ml of medium shaken on a machine working at 180 rpm). In each experiment the growth of the culture (growth of the biomass) was checked nephelometrically. The change in the chemical composition of the incubates was determined in the following way: samples of the culture liquid were taken periodically, the bacterial cells were separated by centrifuging, and the supernatant liquid was concentrated in vacuum and subjected to qualitative and quantitative analysis by partition chromatography and electrophoresis on paper. The systems of solvents used are given in the table. The pyrimidine derivatives were revealed on the chromatograms and phoregrams by their absorption of UV light [2]. The barbituric acid and urea were detected with p-dimethylaminobenzaldehyde [3]. The identification of the individual components was carried out by direct comparison with authentic reference samples of known structure. Quantitative determinations of the pyrimidine bases were carried out by the direct photometry of the appropriate eluates at fixed wavelengths in the 260–285 nm region.

Using the methods described, we found that in the growth of the mycobacteria on media containing uracil (I) or cytosine (III) two new compounds, A_1 and B, appeared in the culture liquid, while in media containing thymine (II) the above-mentioned compound B and a new compound A_2 were detected. A study of the chromatographic characteristics, electrophoretic mobilities, and UV absorption spectra of compound A_1 permitted its identification as barbituric acid (VIII), while compounds A_2 and B proved to be identical with 5-methylbarbituric acid (IX) and urea (XIV), respectively (see table). Thus, it may be considered as established that under the influence of mycobacteria uracil (I) forms as an intermediate barbituric acid (VIII) which is degraded further with the liberation of urea (XIV). In the case of thymine (II), the product of microbial transformation detected first is 5-methylbarbituric acid (IX) which also undergoes further degradation with the formation of urea (XIV). However, in the case of cytosine (III), microbial transformation begins with its deamination to uracil (I), after which all the subsequent stages take place as described above for uracil.

The results presented show that growing whole cells of mycobacteria effect the transformation of compounds (I–III) according to the general scheme given above of the oxidative catabolism of the pyrimidine bases, which has previously been found mainly in studies on the transformation of pyrimidine bases by cell-free extracts and by purified enzyme preparations of certain microorganisms [4–10].

Under comparable conditions, the incubation of a mycobacterium utilizes thymine (II) most actively, uracil (I) somewhat more slowly, and cytosine (III) still more slowly. The barbituric acid (VIII) and 5-methylbarbituric acid (IX) formed as a result of the first stages of transformation generally do not accumulate in considerable amounts, and independent proofs of the intermediate nature of these substances and their rapid further utilization by the mycobacteria have been obtained in a special series of experiments in which VIII and IX served as the sole sources of carbon and nitrogen.



XIV

I R = H, X = O; II R = CH_3, X = O; III R = H, X = NH; IV R = F, X = O; V R = Cl, X = O; VI R = Br, X = O; VII R = I, X = O; VIII R = H; IX R = CH_3; X R = F; XI R = Cl; XII R = Br; XIII R = I

It must also be mentioned that if additional sources of carbon and readily assimilable nitrogen (for example, ammonium sulfate) are added to synthetic media containing any of the pyrimidine bases (I–III), the oxidative cleavage of the pyrimidine base is greatly retarded and takes place practically only in the stationary phase of the growth of the culture, while under these conditions the conversion of these bases into the corresponding pyrimidine nucleosides begins to predominate over their oxidative transformation. The fact that uridine, thymidine, and cytidine are not used as sources of carbon- and nitrogen-containing nutriment by mycobacteria may be of practical interest.

When mycobacteria are grown on media containing any compound of the group of 5-halouracils (IV-VII) as the sole source of nitrogen, the latter are also subjected to transformation. In experiments with 5-chlorouracil (V) and 5-bromouracil (VI) the intermediate formation of compounds A_4 and A_5 was found, and these compounds have been identified as 5-chlorobarbituric acid (XI) and 5-bromobarbituric acid (XII), respectively (see table). In the case of 5-iodouracil (VII) the intermediate formation in the culture liquid of a new compound A_6 was found, and this proved to be identical with unsubstituted barbituric acid (VIII) (see table). This result is apparently explained by the fact that the 5-iodobarbituric acid (XIII) formed initially rapidly undergoes deiodination. In experiments on the incubation of mycobacteria in media containing 5-fluorouracil (IV) a new compound A_3 was found to accumulate in the culture liquid, and this, judging from its chromatographic, spectral, and electrophoretic characteristics (see table) is also a barbituric acid derivative. Although in this case, because of the absence of a reference sample, it was impossible to identify the substance reliably, it is extremely probable that compound A_3 formed by the microbiological transformation of 5-fluorouracil (IV) is 5-fluorobarbituric acid (X).

On the whole, the results obtained show that 5-halouracils are involved by mycobacteria in processes of oxidative catabolism, and that the transformation of these unnatural compounds of the pyrimidine series takes place by the same routes as in the case of natural pyrimidine bases (see scheme). It must be emphasized, however, that under comparable conditions the reactions of the microbiological transformation of 5-halouracils take place considerably slower and with greater difficulty than the analogous transformation reactions of uracil.

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Chromatographic, Spectral, and Electrophoretic Characteristics of the Compounds Found

Compound	R_f in system*				EM × 10^5 at pH 11 (borate buffer), $cm^2 \cdot V^{-1} \cdot sec^{-1}$	UV absorption, nm			
						pH 1		pH 11	
	1	2	3	4		λ_{max}	λ_{min}	λ_{max}	λ_{min}
Uracil (I)	0.57	0.65	0.61	0.60	+4.1**	259	227	284	241
Thymine (II)	0.70	0.77	0.74	0.72	+3.2	264	233	291	244
Cytosine (III)	0.58	0.60	0.15	0.57	+2.7	277	238	283	251
5-Fluorouracil (IV)	0.55	0.78	0.50	0.70	+4.3	266	231	282	246
5-Chlorouracil (V)	0.62	0.78	0.57	0.70	+7.3	273	237	288	253
5-Bromouracil (VI)	0.63	0.80	0.73	0.72	+6.8	275	241	290	254
5-Iodouracil (VII)	0.67	0.80	0.61	0.78	+5.6	282	246	293	257
Barbituric acid (VIII), A_1 and A_6	0.50	0.42	0.15	0.58	+4.3	254	236	258	240
5-Methylbarbituric acid (IX) and A_2	0.56	0.45	—	—	+4.1	—	—	268	241
A_3 [5-fluorobarbituric acid (X) ?]	0.50	0.42	0.10	0.40	+4.5	268	242	269	243
5-Bromobarbituric acid (XII) and A_5	0.50	0.42	0.15	0.58	+4.3	267	244	271	242
5-Chlorobarbituric acid (XI) and A_4	0.50	0.42	0.23	0.41	+3.8	268	238	269	241

*System 1: isopropanol–25% ammonia–water (14 : 1 : 5); system 2: isopropanol–saturated aqueous solution of ammonium sulfate (2:1); system 3: ethyl acetate–acetic acid–water (3 : 1 : 1); system 4: n-butanol–acetic acid–water (2 : 1 : 1)

** Here and below, the plus sign shows the migration of the substance towards the anode.

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DETERMINATION OF THE ENTHALPY OF THE 1,3-DIPOLAR CYCLOADDITION REACTION OF NITRONE ETHERS

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The enthalpy of the 1,3-dipolar cycloaddition of 2-nitroisoxazoline N-oxide to ethylene has been determined.

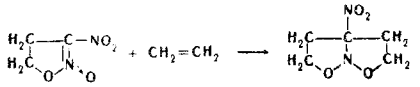
In recent years, the attention of chemists has been attracted increasingly to multicenter processes taking place with the cyclic coordinated transfer of electrons.

Heat of Combustion of 8-Nitroisoxazolizidine*

m	δ	Δt	q′	q″	Q_v
0.19645	0.031	2.391	3.4	7.2	4461.7
0.22519	0.040	2.739	4.0	6.7	4465.1
0.23077	0.041	2.815	4.1	10.6	4462.0
0.23232	0.042	2.833	4.1	9.1	4467.1
0.20484	0.036	2.506	3.8	10.4	4469.3
0.20213	0.036	2.463	3.8	8.8	4458.4

*Q_V = 4463.9±3.2 cal/g; Q_V = 714.8± ±0.5 kcal/mole, ΔH^0 = -29.9±0.5 kcal/mole.

The 1,3-dipolar cycloaddition reaction discovered at the beginning of the sixties by Huisgen [1] is rapidly assuming the leading role in this type of reaction. Important in the study of 1,3-dipolar cycloaddition is the elucidation of the general laws of the reaction and the determination of its position in relation to other multicenter processes. The present work, in particular, took as its object the determination of the enthalpy of the reaction. As a concrete example of the reaction, we selected the 1,3-dipolar cycloaddition of 2-nitroisoxazoline N-oxide to ethylene [2].



In order to determine the heat effect of this reaction the enthalpy of formation of 8-nitroisoxazolizidine was determined from the heat of combustion of this substance found experimentally (the heat of formation of 2-nitroisoxazoline N-oxide has been determined previously, being −12.2 ± 0.5 kcal/mole [3]).

The compound was burned using the semimicrocalorimetric method in a calorimeter with an energy equivalent of 371.0 cal/deg [4] (1 cal = 4.1840 J). The temperatures were read on a calorimetric thermometer, 1° of which corresponds to 0.666°C. The combustion was performed on repeatedly recrystallized samples obtained by two syntheses.

The results of the combustion of 8-nitroisoxazolizidine given in the table, where m is the weight of the sample in g; δ is the correction for heat exchange with the calorimeter with an isothermal jacket, deg; Δt is the rise in temperature corrected for heat exchange, deg; q′ is the heat correction for the formation of nitric acid, cal; q″ is the correction for the combustion of the heating wire, cal; Q_V is the heat of combustion of the compound under the bomb conditions, cal/g and kcal/mole; and ΔH^0 is the standard enthalpy of formation of the substance, kcal/mole, calculated with the use of calorimetric corrections (Washburn, et al.) taking into account the heat of formation of the combustion products CO_2 and H_2O, equal to −94.052 and −68.317 kcal/mole, respectively [5].

The heat of formation of 8-nitroisoxazolizidine was found to be −29.9 ± 0.5 kcal/mole.

From the results obtained it is possible to calculate the enthalpy of the 1,3-dipolar cycloaddition of 2-nitroisoxazoline N-oxide to ethylene, using the enthalpies of formation of 2-nitroisoxazoline N-oxide (−12.2 kcal/mole) and of ethylene (+12.5 kcal/mole). The enthalpy of the reaction is +30.2 ± 1.0 kcal.