ENZYMIC SYNTHESIS OF 9- AND 7-(2′-β-D-DEOXYRIBOSYL) XANTHINE

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1. Introduction

Several bacterial species have growth requirements for vitamin B_{12} or a deoxyriboside, free purines and a pyrimidine [1]. Is it a mere coincidence if in one of these bacteria at least, *Lactobacillus helveticus*, a deoxyribosyl transfer enzyme seems to be able to synthesize preferentially an analogue of the pseudo-vitamin B_{12} nucleoside?

The first chemical synthesis of a 7-deoxyribosyl derivative of a purine base recently described by Rousseau et al. [2] prompts us to report the first enzymic synthesis of a derivative of xanthine which we believe to be 7-(2′-deoxy-β-D-ribofuranosyl) xanthine. A partially purified extract of *L. helveticus* contains a very active trans-*N*-deoxyribosylase (EC 2.4.2.6) which transfers the deoxyribosyl moiety from a purine or pyrimidine deoxyriboside (donor) to a purine or pyrimidine base (acceptor) [1]. During the investigation of this transfer it was very regularly noticed that xanthine (X_1) acting as an acceptor gave 2 products easily resolved on thin layer plates. These 2 compounds will be named respectively X_2 and X_3. We have attempted to identify these 2 compounds as reported here.

Abbreviations used:
CM-cellulose: carboxy-methyl-cellulose;
ORD: optical rotary dispersion;
CD: circular dichroism;
MCD: magnetic circular dichroism;
TLC: thin layer chromatography.

2. Experimental

Xanthine, xanthosine, deoxyguanosine, 9-methyl-xanthine and 7-methyl-guanine were purchased from Calbiochem or Sigma and were checked for purity by thin layer chromatography. Xanthine-8-^{14}C and deoxyadenosine-U-^{14}C (randomly labelled) were obtained from the Département des Radioéléments, Saclay. Thymidine labelled in the sugar moiety was prepared in the laboratory by the transfer reaction:

$$\text{thymine} + \text{deoxyadenosine-U-}^{14}\text{C} \rightleftharpoons \text{thymidine}$$

$$(\text{deoxyribose-}^{14}\text{C}) + \text{adenine-U-}^{14}\text{C}$$

catalysed by the trans-*N*-deoxyribosylase.

The trans-*N*-deoxyribosylase (EC 2.4.2.6) was prepared as previously described [3] from *L. helveticus* (NDC 030) grown in the laboratory. The bacterial cells were broken and extracted with a 10^{-1} M phosphate buffer, pH 6.0. The enzyme was precipitated with $(NH_4)_2SO_4$ (between 30 and 70% saturation). Nucleic acids were removed by precipitation with 0.5% protamine sulfate until absorbancy at 260 nm was constant at a low value. The protein fraction was slightly enriched on a CM-cellulose column and used without further purification.

Standard tests were carried out in a 0.01 M phosphate buffer, pH 6.0, at 40°. The reactants xanthine and thymidine were usually in the ratio 1:2–1:5. The products were separated on thin layer plates for

Table 1
Spectral characteristics of xanthine derivatives.

Compound	λ (max_1)	λ (min_1)	λ (max_2)	λ (min_2)	A_{250}/A_{260}	A_{280}/A_{260}	A_{290}/A_{260}
1) Aqueous solutions of pH 6.9:							
Xanthine	–	–	270	240	0.69	0.87	1.13
Xanthosine	249	222	275	264	1.19	0.98	0.55
9-(2′-deoxy-β-D-ribosyl) xanthine	249	222	275	265	1.20	0.98	0.56
X_3	247.5	228	275	265	1.17	0.99	0.60
7-methyl-xanthine	–	–	270	241	0.58	0.96	0.30
1,7-dimethyl-xanthine	–	–	268	242	0.61	0.88	–
X_2	–	–	270	242	0.56	0.91	0.25
2) Aqueous solutions of pH 11.0:							
Xanthine	241	224	278	258	1.40	1.89	–
Xanthosine	248.5	224	278	264.5	1.28	1.14	0.67
9-(2′-deoxy-β-D-ribosyl)xanthine	248.5	224	278	264.5	1.29	1.13	0.68
X_3	247	228	276.5	265	1.24	1.00	0.73
7-methyl-xanthine	–	–	290	255	1.02	3.25	3.80
1,7-dimethyl-xanthine	–	–	289	255	1.2	2.9	3.5
X_2	–	–	291.5	256	1.25	3.5	4.5

analytical purposes or on Whatman III paper for the preparative batches using water, pH 10.0, as the developing solvent.

3. Results and discussion

In a standard test with thymidine as a donor, the yields were 80% of the total products for X_2 and 20% for X_3. When xanthine-8-^{14}C was used as the acceptor, both compounds were radioactive and gave, after purification and subsequent hydrolysis in an acidic medium at room temp, only xanthine as characterized by TLC in 6 different solvents. When thymidine labelled solely on the sugar moiety was used as a donor, both compounds were radioactive. Upon subsequent hydrolysis both gave the same radioactive derivative as 2′-deoxyribose-^{14}C treated under identical conditions. Moreover both X_2 and X_3 were competent donors in a test in which adenine was the acceptor. The identical derivative obtained in both cases was identified as 9-(2′-deoxy-β-D-ribofuranosyl) adenine by TLC and UV spectroscopy. The mass spectra indicated a molecular weight corresponding to a deoxyribosyl derivative of xanthine for X_2 as well as X_3. We consider these observations as fairly good evidence that X_2 and X_3 are deoxyribosyl xanthine isomers.

Compound X_3 has R_f's essentially identical with those of 9-(2′-deoxy-β-D-ribosyl) xanthine obtained by chemical deamination of deoxyguanosine; UV, ORD, CD and MCD spectra are in complete accordance. Our UV spectra are also identical with those of 9-(β-D-ribofuranosyl) xanthine and 9-methyl-xanthine (commercially available). It can be concluded that our compound X_3 is 9-(2′-deoxy-β-D-ribofuranosyl) xanthine, the "natural" deoxyriboside of xanthine.

The structure of the X_2 isomer was determined mainly from the similarity of the UV spectrum of

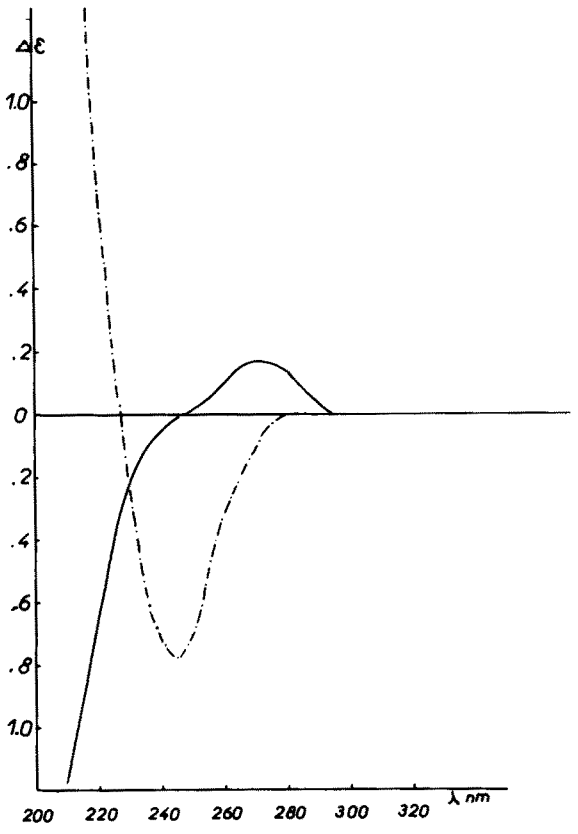

Fig. 1. CD spectra of xanthine derivatives. Phosphate buffer 0.1 M, pH 6.0. X_2: 1.25×10^{-4} M (——). X_3: 0.84×10^{-4} M (– · –).

this compound with that of 7-methyl-xanthine (obtained by chemical deamination of 7-methyl-guanine). Table 1 indicates that 1,7-dimethyl-xanthine has also a very similar spectrum, but the ambiguity was resolved with a derivative prepared from thymidine labelled only in the sugar moiety. The specific radioactivity of the X_2 product was found identical with that of the donor, eliminating the hypothesis of a twice substituted xanthine. We also believe that X_2 is the β anomer on the basis that our enzymic preparation does not transfer the deoxyribosyl group from 9-substituted α anomers (as we mentioned above our X_2 compounds was a competent donor). A preliminary proton NMR study using criteria given by Rousseau et al. [2] brings good evidence that this is indeed the case. Moreover, if X_2 is not the α anomer of X_3, the sign inversion observed in the CD spectrum of compound X_2 (fig. 1) as compared to X_3 is not in contradiction with a certain degree of symmetry between the 2 molecules themselves.

A more extensive investigation is needed to understand the biochemical implications of this finding and the present research is being actively pursued.

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