3-Methylxanthosine

In view of the present-day interest in methylated purines as minor components of RNA and DNA[1], and their possible role in mutagenesis, carcinogenesis[2] and protein synthesis[3], the preparation of *N*-methylated purine nucleosides and nucleotides has attracted increased attention[4-6]. One of the aims of these investigations is to find the reactive sites of the purine bases toward alkylating agents.

With regard to methylation of xanthosine, Bredereck, Haas and Martini[7] recorded the synthesis of 1,3-dimethylxanthosine. Jones and Robins[5] prepared the 7-methyl derivative, indicating the reactivity of the N_7 position. We wish to report evidence for the formation of small amounts of 3-methylxanthosine, among other products, by the reaction of xanthosine with diazomethane. The structure of this compound was demonstrated by hydrolysis to 3-methylxanthine and D-ribose, which were identified by ultra-violet spectra and paper chromatography.

A chilled solution of diazomethane (5·5 mmole in all) in *N*,*N*-dimethylacetamide was added gradually, with stirring in the cold, to a suspension of 500 mg xanthosine in 1 ml. of the same solvent. The resulting clear solution was added to 50 ml. of cold chloroform. A precipitate formed, which was washed with cold chloroform and dried *in vacuo* (234 mg crude product).

Chromatography of an aqueous solution of the precipitate (25 mg) on a 'Dowex-1-acetate' (× 8,200 mesh) column (2·5 × 66 cm) was carried out by developing and eluting with water. Collection of fractions (4·5 ml., 0·5 ml./min) began immediately after charging the column. The profile of the recorded absorbance at 260 mμ exhibited three peaks. The third peak, comprising fractions 113–150 (maximum at fraction 124), contained the presumed 3-methylxanthosine. Fraction 130 showed the absorption characteristics indicated in Fig. 1. The spectrum is similar to that reported by Pfleiderer and Nübel[8] for 3,9-dimethylxanthine, in analogy to the similarity of the spectra of xanthosine and 9-methylxanthine[8].

Hydrolysis of adjacent fraction 129, by acidifying to *p*H 1 with hydrochloric acid and heating for 2 h at 100°, revealed an ultra-violet spectrum corresponding to that of authentic 3-methylxanthine (Table 1). The identity of the hydrolysate with 3-methylxanthine was further confirmed by R_F values in paper chromatograms. The summit of the third peak, which comprised a pool of fractions 120–128 containing the purest material, was concentrated to dryness *in vacuo* at room temperature. Half the dry material was hydrolysed in 0·1 N hydrochloric acid by heating for 2 h at 100°. The hydrolysate was concentrated *in vacuo* to a small volume, applied to Whatman No. 1 paper, and chromatographed in the following solvent systems: (I) ascending: *n*-butanol/acetic acid/water = 12 : 3 : 5 (v/v)[9]; (II) descending: *n*-butanol/*N*,*N*-dimethylacetamide/water = 2 : 1 : 1 (v/v); (III) descending: *n*-butanol/5 N acetic acid = 2 : 1 (v/v)[8] (Table 2).

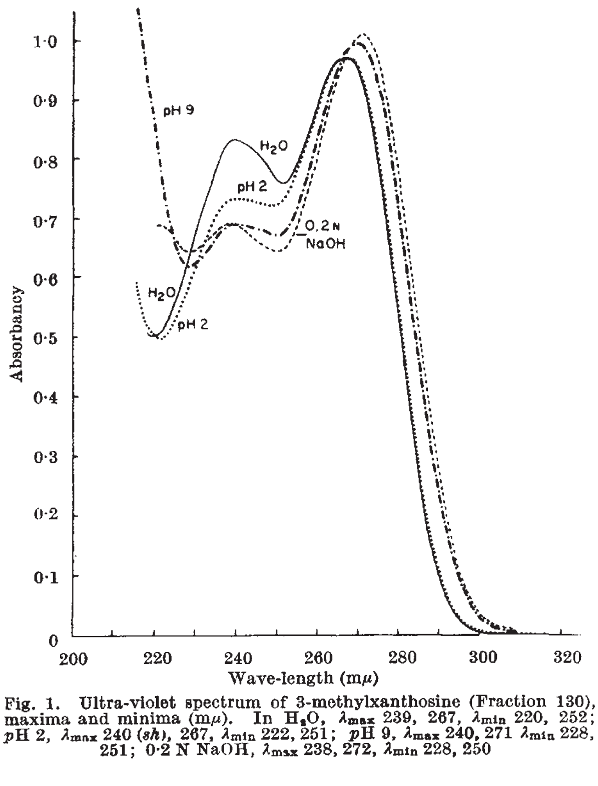

Fig. 1. Ultra-violet spectrum of 3-methylxanthosine (Fraction 130), maxima and minima (mμ). In H_2O, λ_{max} 239, 267, λ_{min} 220, 252; *p*H 2, λ_{max} 240 (*sh*), 267, λ_{min} 222, 251; *p*H 9, λ_{max} 240, 271 λ_{min} 228, 251; 0·2 N NaOH, λ_{max} 238, 272, λ_{min} 228, 250

Table 1. Comparison of Ultra-violet Spectra of Hydrolysed Fraction 129 with that of Authentic 3-Methylxanthine

	Hydrolysate (mμ)		Authentic sample (mμ)	
	Max.	Min.	Max.	Min.
*p*H 1	269	243	270	242
*p*H 9	271	245	272	245
0·2 N NaOH	273	235 (inflex) 248	273	235 (inflex) 248

The identity of the chromatographic spots obtained from the hydrolysate with 3-methylxanthine was again confirmed by the ultra-violet spectrum of the spot extract. Only one sugar spot was obtained from the hydrolysate, which was located with silver nitrate dipping reagent[9], and found to be identical with D-ribose (Table 2).

Table 2. Comparison of R_F Values of 3-Methylxanthosine Hydrolysate, 3-Methylxanthine, Xanthosine and D-Ribose in Three Solvents

R_F values	Solvents I	II	III
Authentic 3-methylxanthine	0·42	0·57	0·43
3-Methylxanthine in hydrolysate	0·43	0·55	0·44
Xanthosine	0·22	0·38	
1,3-Dimethylxanthine			0·67
Authentic D-ribose	0·33		0·28
D-Ribose in hydrolysate	0·33		0·29

Concentration of the eluate pool of fractions 120–128 was associated with considerable hydrolysis, reflected in a complete change of the ultra-violet spectrum and the appearance of spots on paper chromatograms indicating the presence of 3-methylxanthine and D-ribose.

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BIOCHEMISTRY

Effect of Amitosis on the Distribution of Human Lactic Acid Dehydrogenase Isozymes

The lactic acid dehydrogenase (LDH) of mammalian tissues exists as five isozymes[1] which have different charges, structures and biochemical properties[2]. The pattern of isozyme distribution in embryonic and adult