

THE REACTION OF ACRYLIC ANHYDRIDE WITH
ISOPROPYLDENEINOSINE

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(Received in UK 22 March 1968; accepted for publication 1 April 1968)

5'-O-Acryloyluridine has been obtained by the action of acrylic anhydride in pyridine on isopropylideneuridine followed by removal of the isopropylidene group and 5'-O-acryloylthymidine was obtained by selective acylation of thymidine. Both compounds were copolymerized with acrylamide to give a series of water-soluble copolymers, a portion of which was shown to interact with denatured deoxyribonucleic acid (1). Attempts to obtain 5'-O-acryloyl esters of purine nucleosides by similar procedures have given a complex mixture of products.

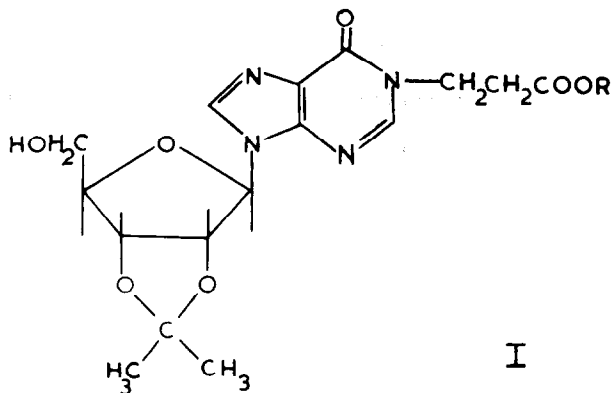
Isopropylideneinosine (600mg.) was dissolved in dry dimethylformamide (12ml.) and dry pyridine (0.5ml.) and acrylic anhydride (0.25ml.) added. After 5hr. at room temperature all of the starting material had been consumed and four major ultraviolet-absorbing components were produced, as shown by paper chromatography in butan-2-ol:water (7:5, organic phase). None of these had the properties expected of simple acryloyl esters and by paper electrophoresis at pH 6.8 it was established that three of the components A, B and C were acids. The fourth component had a typical 'pyridine-like' ultraviolet absorption spectrum and was probably a complex resulting from the reaction between acrylic acid and pyridine. On treatment of the reaction mixture with cold dilute alkali (2) this component was decomposed. The ultraviolet absorption spectra of A, B and C were typical of 1:9-disubstituted hypoxanthines and it appeared that these products had been formed by alkylation of the purine ring to give carboxyethyl derivatives. The results are listed in Table 1.

TABLE 1

	$\lambda_{\text{max.}}$ (m μ)		R_F (butan-2-ol:H ₂ O)	Mobility (cm/hr) pH 6.8, 1 Kv.
	pH 1	pH 11		
A	251	251 (sh. 247;270)	O. 43	4.0
B	252	251 (sh. 270)	O. 49	2.6
C	251	252 (sh. 270)	O. 55	2.0

A was the major component but could not be effectively separated from B and C. It was, however, obtained chromatographically pure by elution from paper chromatograms. Acidic hydrolysis of A (50% HCOOH, 5 hr. reflux) gave D, which had R_F 0.38 in butan-2-ol:water and mobility 4.4 cm/hr. towards the anode on paper electrophoresis at pH 6.8. The ultraviolet absorption spectrum of an eluate of D had $\lambda_{\text{max.}}$ 249 m μ (pH 1); 251 m μ (pH 5.5); 260 m μ (pH 11).

It has been shown by Yoshida and Ukita that acrylonitrile reacts with inosine to give 1-cyanoethylinosine (3). We have converted this into 1-cyanoethylhypoxanthine by acidic hydrolysis. The product had m. p. 246-247° (decomp.) and elemental analysis gave C, 50.86; H, 3.84; N, 37.60 (C₈H₇N₅O requires C, 50.90; H, 3.70; N, 37.00%); ultraviolet spectra, $\lambda_{\text{max.}}$ 249 m μ (pH 1), $\epsilon = 10,700$; 251 m μ (pH 5.5); 260 m μ (pH 11); infrared spectra (KBr disc), $\nu_{\text{max.}}$ 2266 cm⁻¹ (C \equiv N). Treatment of 1-cyanoethylhypoxanthine with 98% formic acid at 175° for 1 hr. (sealed tube) gave as a major product, 1-carboxyethylhypoxanthine, which was identical in all respects to D. This indicated that A was 1-carboxyethyl-2', 3'-O-isopropylideneinosine (I : R = H).



This was confirmed by treatment of isopropylideneinosine (lg.) under reflux with methyl acrylate (10 ml.) and dimethylformamide (50 ml.) for 2 hr. The solvent was evaporated off to give a clear syrup, which was crystallized from chloroform and light petroleum (b. p. 60-80°) to give 1-carbomethoxyethyl-2', 3'-O-isopropylideneinosine (I : R = CH₃) as white needles (700 mg.), m. p. 127-128°. Elemental analysis gave C, 51.90; H, 5.79; N, 13.96 (C₁₇H₂₂N₄O₇ requires C, 51.70; H, 5.60; N, 14.20%); ultraviolet spectra, λ_{max} , 251 m μ (pH 1), ϵ = 10,140; 251 m μ (sh. 270m μ) (pH 11); infrared spectra (KBr disc) ν_{max} , 1740 cm⁻¹ (CO.OMe), 1680 cm⁻¹ (C = O).

Alkaline hydrolysis of I (R = CH₃) gave I (R = H), m. p. 133-134°. Elemental analysis gave C, 50.11; H, 5.38; N, 14.63 (C₁₆H₂₀N₄O₇ requires C, 50.53; H, 5.28; N, 14.73%); ultraviolet spectra, λ_{max} , 251 (pH 1), ϵ = 10,500; 251 m μ (sh. 270 m μ) (pH 11). This component was identical to A as shown by its chromatographic, electrophoretic and spectroscopic properties. Furthermore, treatment of I (R = CH₃) under reflux with 50% formic acid for 5 hr. gave 1-carboxyethylhypoxanthine, m. p. 258-259° (decomp.). Elemental analysis gave C, 46.25; H, 4.18; N, 26.93 (C₈H₈N₄O₃ requires C, 46.10; H, 3.85; N, 26.95%). This compound was identical to D and the product obtained from 1-cyanoethylhypoxanthine after acidic hydrolysis. Compound A was therefore 1-carboxyethyl-2', 3'-O-isopropylideneinosine.

The structures of the minor components B and C were not determined. When

pyridine was omitted from the reaction mixture only small amounts of B and C were observed and the formation of A was very much slower. When the reaction was carried out in chloroform, with anhydrous potassium carbonate as base, A was the major product and no C was produced. When acryloyl chloride was used instead of acrylic anhydride, similar reactions were observed in each case. Under none of these conditions was there any evidence for the formation of 5'-O-acryloyl-2', 3'-O-isopropylideneinosine.

The authors thank Professor M. Stacey for his interest and the Medical Research Council for financial assistance.

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