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The reaction of β-propiolactone with guanosine, deoxyguanylic acid and RNA

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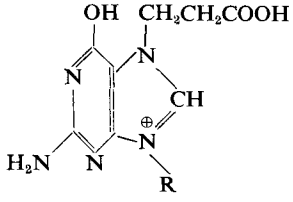
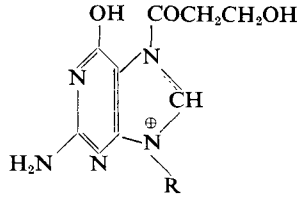
β-PROPIOLACTONE is a compound of considerable interest since it was shown to be mutagenic by Smith and Srb[1] in *Neurospora*, and was later shown to be a complete carcinogen for mouse skin by Roe and Glendenning[2] and Searle,[3] and was also found by Dickens and Jones[4] to produce sarcomas at the site of injection in rats. The latter authors[4] showed also that the compound reacts readily with cysteine at physiological pH yielding S-(2-carboxyethyl) cysteine.

Roberts and Warwick[5] reported briefly on the interaction of β-propiolactone with DNA, deoxyguanylic acid and guanosine *in vitro* and indicated that the products were derived from reaction at the N-7 nitrogen of the guanine moiety. The lactone is a highly strained molecule which, under the appropriate conditions, reacts readily by ring opening. This can occur in two ways yielding, in the case of amines, both amides and amino acids as shown by Gresham *et al.*[6] The relative yields of the products from the two competing reactions

have been found to vary with the amine, the solvent and the order of addition. In the case of the tertiary amines studied by Gresham *et al.* however, formation of the amino acid occurred exclusively. In view of the findings of Lawley[7] and of Brookes and Lawley[8] that the 7-nitrogen atom of the guanine moiety in nucleic acids, nucleosides or nucleotides is highly reactive towards alkylating agents of the haloalkyl type, it seemed likely that this position would also be prone to attack by β-propiolactone. Further, since this atom is a tertiary nitrogen atom one would predict on the basis of Gresham's work that the most likely product formed from its interaction with β-propiolactone would be the acid I and not the amide II.



I



II

Analytical and spectral data has indicated that reaction at the N-7 position does occur in the case of guanosine and deoxyguanylic acid. The guanine in RNA reacts in the same way and the hydrolysis product is similar in all respects to 7-(2-carboxyethyl)guanine prepared unambiguously by the reaction of β-iodopropionic acid with deoxyguanylic acid.

MATERIALS AND METHODS

β-Propiolactone was obtained from L. Light & Co., and purified by redistillation. β-Iodopropionic acid was obtained from the British Drug Houses Ltd. Absorption spectra were measured with a Unicam S.P. 500 spectrophotometer. Paper chromatography was carried out on Whatman No. 1 filter paper using the following solvents by upward flow chromatography.

(1) Methanol–concentrated hydrochloric acid–water (7:2:1);
(2) Butan-1-ol–aqueous ammonia (d. 0·88)–water (86:2:12);
(3) Ethanol–water–aqueous ammonia (d. 0·88) (80:18:1).

Absorption spectra and R_f values of 7-(2-carboxyethyl)guanine

Solvent	λ_{max}(mμ)	$10^{-3}\epsilon$	λ_{min}(mμ)	$\frac{\epsilon_{280}}{\epsilon_{260}}$
$\frac{N}{10}$ HCl	250, 270*	10·1, 6·9	230	0·73
$\frac{N}{10}$ NaOH	280	7·4	257	1·8
0·1M Na Phosphate buffer pH 7	283, 245*	7·2, 5·8	260	1·8

* inflection

Solvent	R_f *Value*
1	0·46
2	<0·1
3	0·45

EXPERIMENTAL

The Reaction of β-Propiolactone with deoxyguanylic acid

β-Propiolactone (120 mg) was added to a solution of deoxyguanylic acid (120 mg) in 0·1M phosphate buffer (pH 7·2) and the mixture kept at 37° for 24 hr. The precipitate (20 mg) was collected and recrystallized from water giving fine needles of 7-(2-carboxyethyl)guanine monohydrate, m.p. >340°.

Found C = 40·2, H = 4·5, N = 29·2%

$C_8H_{11}O_4N_5$ requires C = 39·8, H = 4·6, N = 29·0%

(Loss in weight at 120° was 8·4%; required 7·5%).

For anhydrous material:

Found C = 43·6, H = 4·3%

$C_8H_9O_3N_5$ requires C = 43·1, H = 4·1%.

Reaction of β-propiolactone with guanosine

Guanosine (0·46 g) was suspended in water (4·5 ml), treated with β-propiolactone (0·57 g) and the mixture gently warmed. Within 3 min complete solution was attained (pH = 6). After a further 5 min at room temperature the mixture was treated with an equal volume of 2N HCl and heated for 1 hr at 100°. The cooled solution was neutralized by the dropwise addition of aqueous ammonia yielding 0·32 g of crude 7-(2-carboxyethyl)guanine monohydrate (0·15 g after recrystallization from water).

Reaction of β-iodopropionic acid with deoxyguanylic acid

Deoxyguanylic acid (120 mg) in 4M phosphate buffer pH7 (5 ml) was treated with β-iodopropionic acid (225 mg) which had been neutralized with caustic soda, and the mixture kept at 37° for 48 hr. The precipitated solid (20 mg) was recrystallized from water and shown to be similar to 7-(2-carboxyethyl)guanine in its physical characteristics (analysis, R_f in solvents I, II, III and absorption spectrum in water, acid and alkali).

Reaction of β-propiolactone with yeast RNA

Yeast RNA (1·2 g) in 0·2M phosphate buffer pH 7·2 (30 ml) was treated with β-propiolactone (250 mg), and the mixture kept at 37° for 48 hr. The RNA was precipitated by the addition of ethanol (60 ml) and sodium acetate to 2%, centrifuged, washed several times with ethanol, dried, and hydrolysed with 1N HCl (5·5 ml) for 1 hr at 100°. The solution was cooled and the 7-(2-carboxyethyl)-guanine precipitated by the careful addition of NaOH solution. The yield was 11 mg. After purification, physical properties confirmed that the compound was 7-(2-carboxyethyl)guanine monohydrate.

Paper chromatography of the mother liquors from the reaction mixture confirmed the almost complete absence of guanine.

Chester Beatty Research Institute,
Institute of Cancer Research, Royal Cancer Hospital,
*Fulham Road, London, S.W.*3.

J. J. Roberts
G. P. Warwick

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