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STUDIES OF THE REACTION OF β-PROPIOLACTONE WITH DEOXYGUANOSINE AND RELATED COMPOUNDS*

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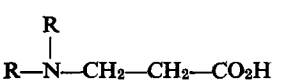
Abstract—The reaction of β-propiolactone with guanosine and related compounds was shown to give the previously reported product 7-(2-carboxyethyl)guanine, and a second product which was characterized as 7,9-di-(2-carboxyethyl)guanine. Evidence for this structure included an elementary analysis of the compound, comparison of its u.v. spectra and p*K*a with those for analogous structures, and comparison of its spectra and R_F with those for the compound prepared by an alternative method. A mechanism for the reaction of β-propiolactone with guanosine and related compounds is suggested.

β-PROPIOLACTONE is of considerable biological interest. It is useful as a sterilizing agent,[1] it is mutagenic for Neurospora,[2] and it causes skin tumors in mice[3, 4] and sarcomas in rats.[5] A single application of β-propiolactone to mouse skin initiates the process of tumor formation,[6] showing that a single pulse of the lactone interacts with one or more tissue components.

In-vitro studies have revealed that the lactone is an effective alkylating agent. Dickens and Jones[5] reported alkylation of cysteine by β-propiolactone to form S-(2-carboxyethyl)cysteine. Gresham *et al.*[7] showed that β-propiolactone reacts with primary and secondary amines to form an acid (I) and an amide (II);



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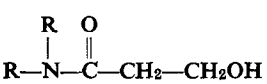

II

but with the tertiary amines Gresham studied, only the acid (i.e. the carboxyethyl) derivative was formed. Roberts and Warwick found that β-propiolactone reacted readily with guanosine, deoxyguanylic acid, and RNA to form the N-7 alkylated product, 7-(2-carboxyethyl)guanine (CEG).[8] By using a reaction time longer than that of Roberts and Warwick, an additional compound characterized by bright blue fluorescence under u.v. light was found among the reaction products of β-propiolactone with guanine by Richardson and Boutwell.[6] Further studies reported here indicate that alkylation occurs at the N-9 as well as the N-7 position to form 7,9-di-(2-carboxyethyl)guanine (DCEG).

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EXPERIMENTAL AND RESULTS

β-propiolactone was obtained from Eastman Organic Chemicals, Distillation Products Industries. It was analyzed quantitatively by the method of Tyler and Beesing[9] and was found to have greater than 95% purity.

Paper chromatography; spectroscopy

Paper chromatography was carried out on Whatman 4 filter paper with the following solvents by ascending chromatography: (1) methanol:concentrated hydrochloric acid:water (7:2:1); (2) isoamyl alchohol: 5% aqueous Na_2HPO_4 (1:1); (3) methanol: ethanol:concentrated hydrochloric acid:water (50:25:6:19); (4) ethanol: water:ammonia (d. 0·88) (80:18:1).

Ultraviolet absorption spectra were measured with a Beckman DB recording spectrophotometer.

Reaction of β-propiolactone with deoxyguanosine

Deoxyguanosine (0·5 g) was suspended in water (5·0 ml), treated with β-propiolactone (2·0 ml, 2·28 g), and the mixture gently warmed to about 50°. Within 3 min complete solution was attained (pH 5). After a further 35 min at room temperature with occasional swirling, the deoxyriboside was hydrolyzed by adding an equal volume

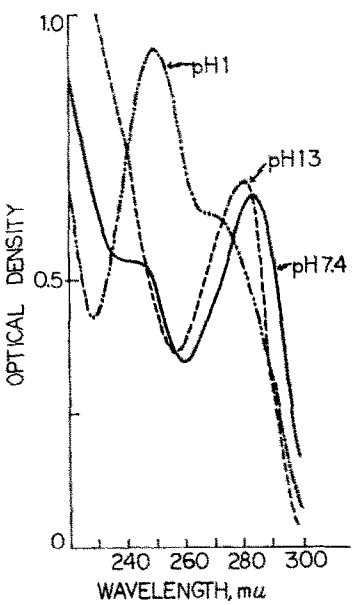


FIG. 1. Absorption spectra of 7-(2-carboxyethyl)guanine (0·0205 mg/ml) at pH 1, pH 7·4 and pH 13; 25°.

of 2 N HCl and heating for 1 hr at 100°. The cooled solution was neutralized by dropwise addition of concentrated ammonia. The precipitate formed was identified as 7-(2-carboxyethyl)guanine by comparison of u.v. spectral and paper chromatographic data with values reported by Roberts and Warwick.[8] (See Fig. 1 for CEG spectra.)

After removal of the precipitate, analysis of the supernatant fraction by paper chromatography and u.v. spectroscopy indicated the presence of a compound other

than CEG. The supernatant was dried on a rotary evaporator, dissolved in 5 ml of water and kept in the cold (5°) for 2 hr to allow precipitation of traces of CEG. The precipitate was removed, and the supernatant dried.

The product was collected and recrystallized from 50% acetone–water, and dried with acetone, giving the crystalline compound 7,9-di-(2-carboxyethyl)guanine. Yield, 300 mg. Found: C = 44·22, H = 4·78, N = 23·39, for anhydrous material. $C_{11}H_{14}O_5N_5$ requires C = 44·4, H = 4·8, N = 23·7. This analysis was obtained after drying *in vacuo* at 120°.

Reaction of β-propiolactone with deoxyguanlyic acid and guanosine

β-Propiolactone (1 ml, 1·14 g) was added to a solution of deoxyguanylic acid (200 mg) in 0·15 M phosphate buffer at pH 7·4 (2 ml) and the mixture kept at 37° for 24 hr. The precipitate was removed and the supernatant yielded DCEG, as verified by spectral and R_F data (cf. Tables 1, 2; Fig. 2).

TABLE 1. ABSORPTION SPECTRA OF 7,9-DI-(2-CARBOXYETHYL)GUANINE

pH	Max (mμ)	Min (mμ)	$\epsilon_{280}/\epsilon_{260}$
1	254, 280	229, 271	0·70
7·0	254, 280	234, 270	0·82
13	249, 281	242, 262	1·5

TABLE 2. R_F VALUES OF 7,9-DI-(2-CARBOXYETHYL)GUANINE AND OF 7-(2-CARBOXYETHYL)GUANINE

Solvent*	R_F Values for DCEG	Ratio $\frac{R_F \text{ DCEG}}{R_F \text{ Guanine}}$	R_F Values for CEG
1	0·71	2·8	0·46†
2	0·88	1·7	0·62
3	0·58	2·3	
4	0·09	0·27	0·45†

* See text for solvent systems.
† See Ref. 8.

One gram of guanosine was dissolved by warming in 50 ml 0·15 M phosphate buffer at pH 7·4. β-Propiolactone (3·0 ml) was added, and the solution was allowed to stand at room temperature for 2·5 hr. The riboside was hydrolyzed by adding an equal volume of 2 N HCl and heating 1 hr at 100°. After neutralization, the precipitate was removed and the supernatant yielded DCEG as verified by spectral and R_F values.

Reaction of 7-carboxyethylguanine with β-propiolactone

The precipitate from the reaction of guanosine with β-propiolactone, followed by hydrolysis, was dissolved in hot phosphate buffer (pH 7·4), reprecipitated, dried with acetone, and verified to be 7-(2-carboxyethyl)guanine by comparison with spectral and chromatographic data in the literature.[8] To 4 ml of an aqueous suspension of

7-carboxyethylguanine (50 mg, 0·2 μmole) was added β-propiolactone (0·5 ml, 7·8 μmoles). The material dissolved to give a solution with a final pH of 3. The reaction mixture was kept at 37° overnight. The solution was neutralized with ammonia, kept in the cold for 2 hr, and all traces of precipitate were removed. The supernatant yielded DCEG, as confirmed by u.v. spectral and chromatographic data.

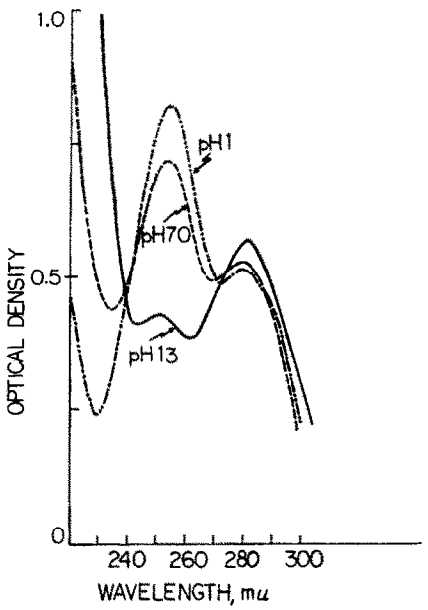


FIG. 2. Absorption spectra of 7,9-di-(2-carboxyethyl)guanine (0·0180 mg/ml) at pH 1, pH 7·0, and pH 13; 25°.

DISCUSSION

Spectral and R_F data for the compound identified as 7,9-di-(2-carboxyethyl)guanine from the reaction of β-propiolactone with deoxyguanosine were identical with those obtained for the compound prepared alternatively by the reaction of β-propiolactone with 7-carboxyethylguanine.

The u.v. spectrum of DCEG was similar to that obtained by Brookes and Lawley[10, 11] for the analogous compound 7,9-di-(2-hydroxyethyl)guanine, and by Lawley and Brookes[12] for the compound 7-methyl-9-ethylguanine. The similarities in the spectral data are consistent with a guanine substituted in positions 7 and 9.

The wavelength maxima of the DCEG spectra were not altered over the pH range 7–1, showing the absence of a basic p*K*a in this region. (A basic p*K*a does occur for CEG.) A p*K*a determination (Fig. 3) showed the presence of a single p*K*a at pH 7·3. This gives further verification of structure by eliminating as possible structures both the 1,7 dialkyl-, and the 7 alkyl-2-alkylamine guanine derivatives which would both have a basic p*K*a around 3. The compounds analogous to DCEG, viz. 7-methyl-9-ethylguanine and 7,9-di-(2-hydroxyethyl)guanine, have also been reported in the literature as having a single p*K*a at 7·3.[12]

Findings in this paper indicate the formation of the mono- and dialkylated products CEG and DCEG when β-propiolactone is reacted with a nucleoside or nucleotide of guanine.

On repeating the experiment of Roberts and Warwick, i.e. the reaction of β-propiolactone and guanosine[8] (8-min reaction time), the only product recovered was CEG.

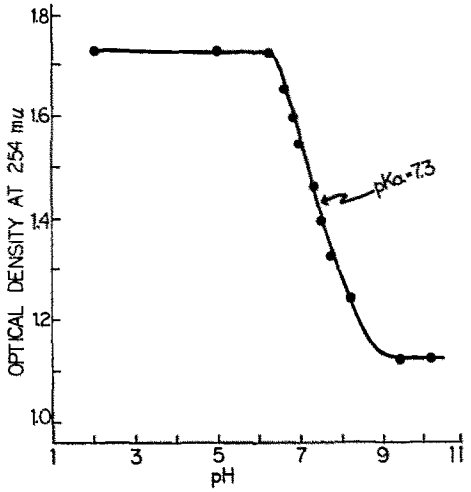
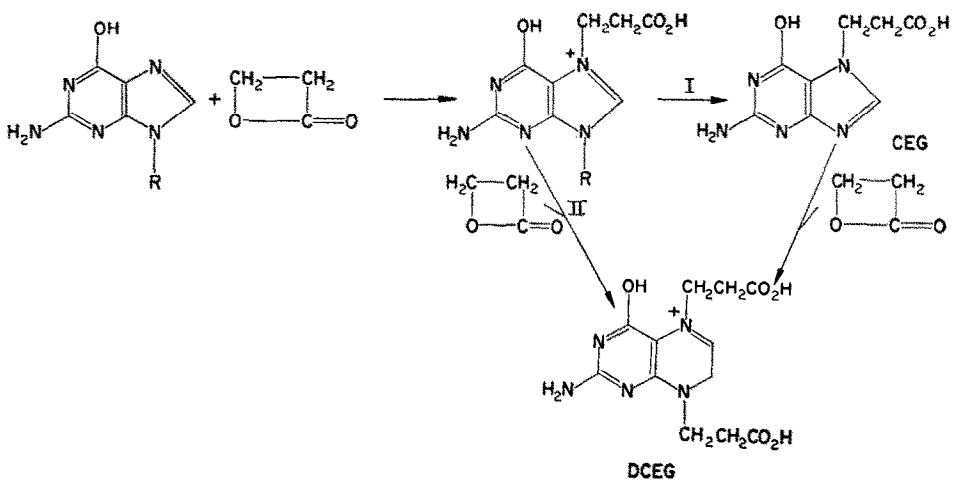


FIG. 3. p*K*a Determination for 7,9-di-(2-carboxyethyl)guanine (0·0327 mg/ml). A single p*K* at 7·3 was found.

DCEG was obtained from guanosine and deoxyguanosine only with longer reaction times. This indicates that the CEG or its riboside or deoxyriboside is the product first formed. The DCEG must form from CEG or a CEG nucleoside.

The reaction of guanosine or deoxyguanosine and β-propiolactone might then be formulated as follows:



(R = ribose, or deoxyribose)

On hydrolysis of the reaction mixture, the glycoside bond of the CEG nucleoside would be cleaved to yield CEG. Any CEG already formed and the DCEG are stable to HCl hydrolysis. When –R is deoxyribose phosphate, i.e. when deoxy-GMP is used, DCEG and CEG are formed without the requirement for HCl hydrolysis.

For the reaction of β-propiolactone with guanosine or deoxyguanosine, two pathways are suggested for the formation of DCEG from the CEG nucleoside. The first (I) is shown in two steps; the alternative (II) involves a one-step SN2 mechanism. Either mechanism would be consistent with the recovery of CEG (from the hydrolysate) as the only product when the reaction time is short. Either mechanism would be consistent with the formation of both CEG and DCEG if longer reaction times are used. Work reported here does not rule out either mechanism.

Biological significance of 7,9-di-(2-carboxyethyl)guanine remains to be investigated.

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