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ACKNOWLEDGMENTS AND ADDRESSES

Received November 28, 1973, from the **Department of Pharmaceutical and Nutritional Sciences, University of Ferdowsi, Meshed, Iran,* and the [1]*Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60680*

Accepted for publication September 26, 1974.

Abstracted in part from a dissertation submitted by S. Javidan to the Graduate College, University of Illinois at the Medical Center, Chicago, Ill., in partial fulfillment of the Doctor of Philosophy degree requirements.

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Transformation of 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine Induced by Hydrogen Peroxide

KIYOMI KIKUGAWA

Abstract □ 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (I) is a more potent and less toxic antineoplastic agent than is cytarabine (1-β-D-arabinofuranosylcytosine) (II). The anhydronucleoside (I) was found to be readily transformed by hydrogen peroxide into 2,2′-anhydro-5-hydroxy-1-β-D-arabinofuranosylcytosine (III) by treatment with 0.025 *M* hydrogen peroxide at a neutral and slightly basic pH range (pH 6–9) and at room temperature. It was converted into non-UV-absorbing substance(s) by hydrogen peroxide at an alkaline pH (pH 11). Since hydrogen peroxide is produced by redox reactions in all living cells, it may be responsible for the alteration of I. Such transformations by hydrogen peroxide were not observed with cytarabine.

Keyphrases □ 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine—hydrogen peroxide-induced transformation, compared to cytarabine reaction □ Hydrogen peroxide-induced hydroxylation—reactions with anhydronucleoside 2,2′-anhydro-1-β-D-arabinofuranosylcytosine and cytarabine □ Antineoplastic agents—hydrogen peroxide-induced transformation of 2,2′-anhydro-1-β-D-arabinofuranosylcytosine, compared to cytarabine

2,2′-Anhydro-1-β-D-arabinofuranosylcytosine[1] (I) is a more potent and less toxic antineoplastic agent than is cytarabine[2] (1-β-D-arabinofuranosylcytosine) (II) with experimental tumors (1–5). Evidence of the clinical effectiveness of this anhydronucleoside against acute leukemia was recently documented (6). Although the anhydronucleoside was first prepared by Walwick *et al.* (7), its chemical properties have not yet been sufficiently investigated.

The anhydro linkage of this nucleoside, although stable in acid, is hydrolyzed in solutions of increased hydroxyl-ion concentration to afford II (7, 8). Other chemical transformations of this anhydronucleoside under the possible biological conditions have not been reported. This paper deals with the transforma-

[1] Cyclocytidine.
[2] Previously named cytosine arabinoside or arabinosylcytosine.

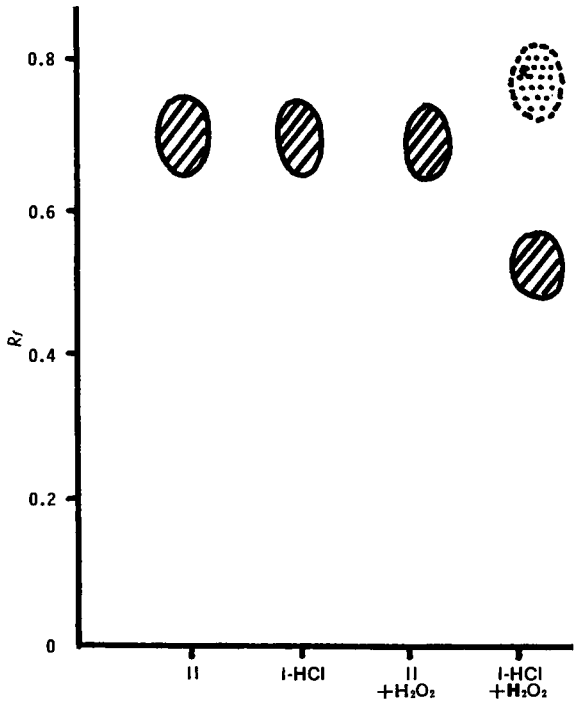


Figure 1—*Paper chromatography of I-HCl and II with 3% hydrogen peroxide. Solvent system was isopropanol–concentrated ammonia–water (7:1:2). Spots were detected by UV radiation and Ehrlich's reagent. Key: ⊘, UV absorbing spot; and ⊛, Ehrlich's reagent positive spot.*

tions of the anhydronucleoside when treated with hydrogen peroxide, since its ubiquitous occurrence in aerobic biological systems makes the reaction of considerable interest.

EXPERIMENTAL[3]

2,2′-Anhydro-1-β-D-arabinofuranosylcytosine (I) Hydrochloride—Compound I-HCl was prepared according to the modified method reported previously (13). Phosphorus oxychloride (33 ml, 0.36 mole) was placed in 200 ml of anhydrous dimethylformamide, and the mixture was set aside at room temperature for 30 min. To the solution was added 10.0 g (41 mmoles) of cytidine, and the mixture was stirred at room temperature for 3 hr and then poured into 150 ml of water to destroy the reagent. The aqueous reaction mixture was allowed to stand at room temperature for 2 hr and then was evaporated *in vacuo* to about 200 ml.

The solution was seeded with an authentic sample of the hydrochloride of I and stored in a refrigerator overnight. The white needles were separated by filtration, washed with ethanol, and dried. The hydrochloride of I was obtained in a yield of 49% (5.23 g), mp 257–259° dec. Physicochemical properties of this sample were identical with those of the authentic sample purified through ion-exchange columns described previously (13).

Paper Chromatography of I-HCl in Presence of Hydrogen Peroxide—Compound I-HCl (100 mg, 0.38 mmole) was dissolved in 1.0 ml of water, and 0.10 ml (1.30 mmole) of 30% hydrogen peroxide was added. The solution was submitted to paper chromatography and was developed with Solvent 1 for 14 hr. A single spot (R_f 0.47) was detected by UV radiation, and the aqueous extract of the spot showed UV absorption maxima at 286 (pH 1) and 258 and 316 (pH 7 and pH 13) nm. The spot consumed metaperiodate in the sodium metaperiodate–potassium iodide spray test. Another spot (R_f 0.74), colored violet with Ehrlich's reagent, was also detected. When I in the absence of hydrogen peroxide or II in the presence of hydrogen peroxide was submitted to paper chromatography and developed as described, only one spot (R_f 0.61) corresponding to II was detected (Fig. 1). The paper chromatograms of I in the presence of the reagent developed with Solvent System 2 or 4 revealed no other spots than that corresponding to I.

Treatment of Pyrimidine Nucleoside with Hydrogen Peroxide in Buffer Systems—Pyrimidine nucleosides such as I, II, 2,2′-anhydro-1-β-D-arabinofuranosyluracil, 2,2′-anhydro-5-hydroxy-1-β-D-arabinofuranosylcytosine (III), cytidine, and uridine were treated with 0.025 *M* hydrogen peroxide in buffer systems at room temperature. For example, a solution of the I-HCl (50 mg, 0.190 mmole) or II (50 mg, 0.205 mmole), 50 μl (0.65 mmole) of 30% hydrogen peroxide, and 450 μl of water was added to 25.0 ml of each of the following buffers, and the mixture was set aside at room temperature. The buffers used were 1 *M* sodium acetate (pH 4.0, 4.9, 5.5, and 5.9), 1 *M* phosphate (pH 5.8, 6.2, 7.0, and 7.9), and 1 *M* glycine–sodium hydroxide (pH 8.0, 9.0, 10.0, and 11.0).

An aliquot (0.10 ml) of each buffered mixture was added to 10.0 ml of 0.1 *N* NaOH; after 5 min at room temperature, the spectrum of the alkaline solution was recorded. Under the alkaline conditions, unreacted I was completely converted into II, and the product (III) was almost intact. From the spectra, absorbances of I and II treated with hydrogen peroxide were calculated (Figs. 2–4). The amount of hydrogen peroxide remaining in the buffered mixture except at pH 11 was determined by iodometry (Fig. 4).

The reaction of I with hydrogen peroxide at pH 7.9 in the presence of ferric chloride or allyl alcohol was also investigated. Thus,

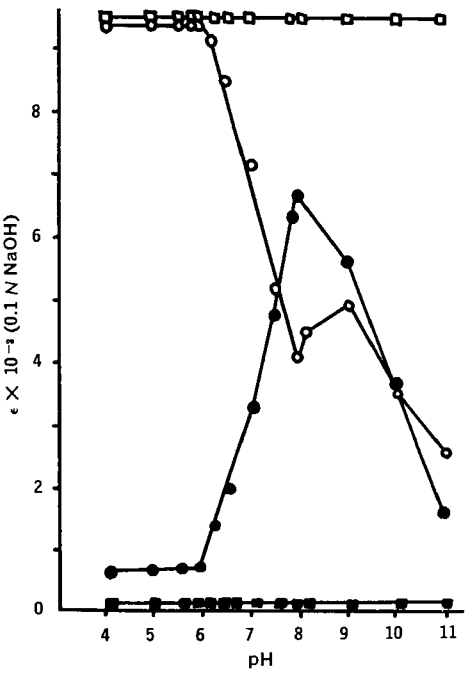


Figure 2—*Changes of UV absorption of I and II treated with hydrogen peroxide under different pH conditions. Compounds I-HCl (7.6×10^{-3} M) and II (8.2×10^{-3} M) were treated with 0.025 M hydrogen peroxide in 1 M buffer systems at room temperature for 18 hr. Absorbance at 275 and 315 nm of the mixture diluted with alkali was measured. Key: ○, I at 275 nm; ●, I at 315 nm; □, II at 275 nm; and ■, II at 315 nm.*

[3] Melting points were determined on a Buchi melting-point apparatus and are uncorrected. UV absorption was measured with a Hitachi recording spectrophotometer, EPS-3T. Optical rotations were obtained with a JASCO polarimeter, DIP-SL. NMR spectra were taken in deuteroxide (internal standard 2,2-dimethylsilapentane-5-sulfonic acid sodium salt) with a Varian-T60 spectrometer. High-resolution mass spectroscopy was measured with a CEC 110B double-focus mass spectrometer.

Paper chromatograms were run on Toyo Roshi No. 51A, using the following solvent systems: 1, isopropanol–concentrated ammonia–water (7:1:2); 2, *n*-butanol–water (84:16); 3, 5 *M* ammonium acetate–0.5 *M* tetrasodium ethylenediaminetetraacetate–saturated sodium borate–ethanol (12:0.3:48:132) (9); and 4, *n*-butanol–acetic acid–water (2:1:1). Paper electrophoresis was carried out in the following buffer systems: A, borate buffer (pH 6.0) (10); and B, 0.05 *M* phosphate buffer (pH 7.5) at 1000 v/35 cm for 1 hr. The spots were detected by UV radiation (253.6 nm) unless otherwise mentioned. The metaperiodate consumption test was done according to the usual method (11). Commercially available 30% hydrogen peroxide was used, and the content of the reagent in the solution was 440 mg (13 mmoles)/1.0 ml, estimated by iodometry. Cytarabine (II) was prepared by the authors (12, 13).

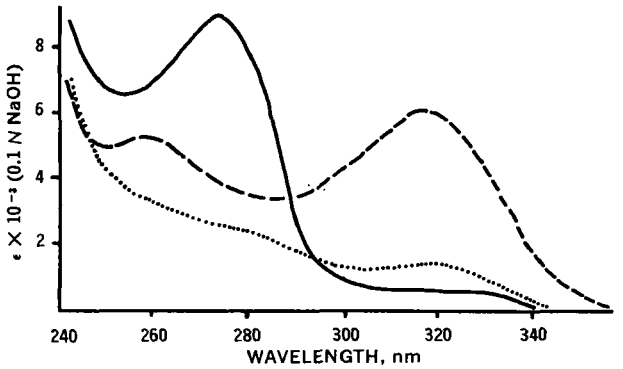


Figure 3—*Full spectra of the reaction mixture of I with hydrogen peroxide diluted with alkali. Conditions were the same described for Fig. 2. Key: —, pH 4.9; — —, pH 7.9; and ....., pH 11.0.*

a solution of the I-HCl (5.0 mg, 0.019 mmole) and 50 μl (0.65 mmole) of 30% hydrogen peroxide in 25.0 ml of 0.1 *M* phosphate buffer (pH 7.9) containing 5×10^{-5} *M* ferric chloride or 5×10^{-2} *M* allyl alcohol was allowed to stand at room temperature. An aliquot (2.0 ml) was diluted with 23 ml of 0.1 *N* NaOH or 0.1 *N* HCl, and the spectrum of the solution was recorded. The effect of ferric chloride and allyl alcohol on the reaction of I with hydrogen peroxide is presented in Fig. 6.

2,2′ - Anhydro-5-hydroxy-1-β-D-arabinofuranosylcytosine (III)—Compound I-HCl (2.00 g, 7.65 mmoles) was added to a mixture of 2.0 ml (26.0 mmoles) of hydrogen peroxide and 1.0 liter of 1 *M* phosphate buffer (pH 7.9) with stirring. The solution was kept at 30° for 18 hr. It was then chilled in an ice water bath, and the crystalline salt was removed by filtration and washed with cold water. The filtrate and washings were combined and treated with 1.0 g of 5% palladium-on-charcoal overnight until free of hydrogen peroxide as detected by potassium iodide-starch paper.

The charcoal was removed by filtration, and the filtrate was acidified to pH 1.0 with concentrated hydrochloric acid and added to a cation-exchange column[4] (250 ml). The column, which had previously been washed with water, was eluted with 250 ml of 3 *N* NH_4OH. The effluent was evaporated at 20° *in vacuo* to dryness, and the residue was triturated with ethanol to afford 1.60 g of a crude crystalline sample of III. Recrystallization from ethanol–water gave 1.20 g of white needles (65.0% yield), mp 275–281° dec., $[\alpha]_D^{25}$ −98.3° (c 0.10, water). The pKa, determined spectrophotometrically, was 5.2. NMR: δ 3.53 (2H, doublet, $H_{5'}$), 4.40 (1H, multiplet, $H_{4'}$), 5.46 (1H, doublet, $H_{2'}$, $J_{1',2'}$ = 6 Hz), 6.54 (1H, doublet, $H_{1'}$, $J_{1',2'}$ = 6 Hz), and 7.00 (1H, singlet, H_6) ppm. High-resolution mass spectroscopy showed a molecular ion peak at *m/e* 241 and an elemental composition corresponding to $C_9H_{11}N_3O_5$.

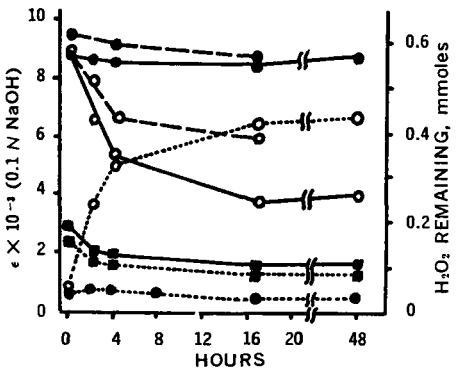


Figure 4—*Time course of the reaction of I with hydrogen peroxide. Compound I-HCl (7.6×10^{-3} M) was treated with 0.025 M hydrogen peroxide at pH 5.0, 7.9, or 11.0 and at room temperature. Absorbance at 275 and 315 nm of the mixture diluted with alkali was measured. The amount of hydrogen peroxide remaining in the mixture was also determined. Key: ●, pH 5.0; ○, pH 7.9; ■, pH 11.0; ——, absorbance at 275 nm; -----, absorbance at 315 nm; and — —, amount of hydrogen peroxide remaining.*

[4] Dowex 50 × 4, free form.

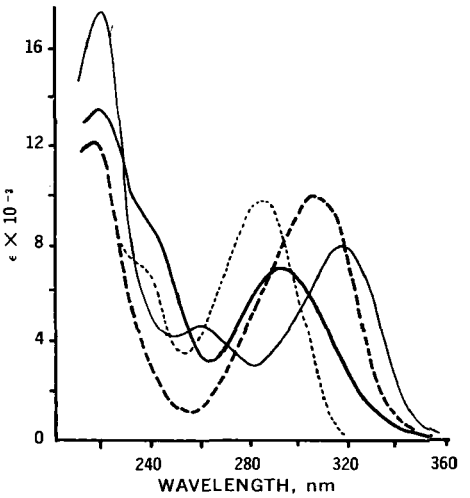


Figure 5—*UV absorption spectra of III treated with 0.1 N NaOH at 50°. Spectra were taken after the mixture was diluted with 0.1 M phosphate buffer (pH 7.0) or 0.1 N HCl. Key: —, for 0 min and then at pH 7.0; ----, for 0 min and then in 0.1 N HCl; ——, for 30 min and then at pH 7.0; and — —, for 30 min and then in 0.1 N HCl.*

Compound III consumed 2 moles of metaperiodate within 30 min and an additional 2 moles over 40 hr. A violet color was produced when III was treated with a 2% ferric chloride solution in water. Data from paper chromatographic, paper electrophoretic, and UV absorption analyses are presented in Table I.

Anal.—Calc. for $C_9H_{11}N_3O_5$: C, 44.81; H, 4.59: N, 17.42. Found: C, 44.68; H, 4.58; N, 17.46.

Alkaline Hydrolysis of III: Spectrophotometric Estimation—Compound III (10.0 mg) was dissolved in 2.0 ml of 0.1 *N* NaOH and incubated at 22 or 50° for 6 hr. An aliquot (50 μl) was diluted with 25 ml of 0.1 *N* HCl or 0.1 *M* phosphate buffer (pH 7.0), and the spectra were recorded. The spectra indicated that III was completely degraded to 5-hydroxy-1-β-D-arabinofuranosylcytosine (IV) at 22° in 6 hr and at 50° in 30 min. Treatment of III in 0.1 *N* NaOH at 50° for an additional 150 min did not show any changes in the spectrum. About 50% of III was transformed into IV at 22° in 65 min, estimated by the absorbance at 320 nm in 0.1 *N* HCl. Representative profiles are given in Fig. 5.

Attempts to Isolate the Hydrolyzed Product (IV)—Compound III (700 mg, 2.9 mmoles) was dissolved in 30 ml of concentrated ammonia and heated at 100° for 5 hr in a sealed tube. Paper chromatography (Solvents 1, 2, and 4) of the mixture revealed a new fluorescent spot detected by UV radiation. The aqueous extract of the spot showed absorption maxima at 304 and 215 (pH 1), 292 and 218 (pH 7), and 321 (pH 13) nm, very close to the maximum (292 nm at pH 7) of the known 5-hydroxycytidine (14). The spot gave a violet color with a 2% ferric chloride solution in water and was not colored by Ehrlich's reagent. The reaction mixture was evaporated *in vacuo* to dryness, and the residue was purified by passage through a cellulose column (1.7 × 50 cm) with Solvent 2 to afford a colorless gum of IV (Table I). All attempts to obtain a crystalline sample of IV failed because it gradually decomposed and darkened on treatment with solvents at room temperature.

RESULTS AND DISCUSSION

During the studies on the chemical transformations of I, it was found that paper chromatography of a solution of I-HCl in hydrogen peroxide with an alkaline solvent system quantitatively converted I into compounds other than its hydrolyzed derivative, II. Thus, paper chromatography of a mixture of I-HCl in hydrogen peroxide developed with an alkaline solvent system revealed one UV-absorbing spot and another non-UV-absorbing spot (Fig. 1). The product (III) corresponding to the UV-absorbing spot differed

Table I—Paper Chromatography, Paper Electrophoresis, and UV Absorption Data of 2,2′-Anhydro-1-β-D-arabinofuranosylcytosine Derivatives

Compound	Paper chromatography (R_f) Solvent System 1	2	3	4	Paper Electrophoresis (Mobility), cm Buffer System A[a]	B	pH	UV Absorption λ_{max}, nm ($\epsilon \times 10^{-3}$)
I·HCl	—	0.08	0.67	0.30	−6.2	−13.5	1–7	263 (10.6), 231(9.5)
II	0.61	0.14	0.66	0.44	−6.2	−7.0	1	282(13.4)[b]
							7	271(9.7)
III	0.47	0.06	0.52	0.44	−6.2	−8.5	1	284 (9.98), 215 (12.2)
							7	316 (8.2), 258 (5.0), 218.5 (17.7)
							13	316 (8.2), 257 (5.0)[d]
IV[c]	0.38	0.15	—	0.37	—	−7.0	1	304 (10.2), 215 (12.2)
							7	292 (7.3), 218 (13.8)
							13	321 (8.0)

[a] Compounds such as cytidine having a *cis*-glycol function at the sugar moiety ran −3.0 cm to cathode. [b] Reference 13. [c] Data of paper chromatography and paper electrophoresis were obtained with the purified gum of IV, and the UV absorption spectrum was obtained by the quantitative transformation of III with 0.1 *N* NaOH at 50°. [d] Measured as soon as possible.

from I or II and had the absorption maxima at 258 and 316 nm (pH 7 and 13). The reaction on the paper chromatogram using the alkaline solvent system was not observed when a mixture of I in water in the absence of hydrogen peroxide or a mixture of II and the reagent was developed. Therefore, I was converted into III and other non-UV-absorbing substance(s) by hydrogen peroxide without prior hydrolysis to II.

To characterize the pH dependence of the reaction of I with hydrogen peroxide, I and II were treated with a 3.4 molar excess of 0.025 *M* hydrogen peroxide in buffer systems (pH 4–11) at room temperature for 18 hr (Fig. 2). Although the absorbances of the mixtures of II and hydrogen peroxide were not altered over the 4–11 pH range, those of the mixtures of I and hydrogen peroxide were altered extensively, depending on the pH of the buffer systems. Thus, the absorbances at 275 nm of the mixtures of I and the reagent were not altered below pH 6 but extensively decreased above pH 6. The absorbances at 315 nm of the mixtures increased over the 6–8 pH range and decreased at more alkaline pH values. Some of the full spectra of the reaction mixtures are shown in Fig. 3. Figures 2 and 3 indicate that I reacted with hydrogen peroxide in two fashions without prior hydrolysis to II. At around pH 8, I was mainly converted into III, having an absorption maximum at 316 nm (pH 13). At around pH 11, I was mainly converted into non-UV-absorbing substance(s).

A time course study of the reaction of I with hydrogen peroxide is presented in Fig. 4. At pH 5.0, little reaction occurred over 48 hr, and little hydrogen peroxide was consumed during the first 17 hr. At pH 7.9, both the decrease of the absorbance at 275 nm and the increase at 315 nm indicated that the formation of III gradually proceeded, reaching a maximum at 17 hr. About one-third of the 3.4 equimolar amount of hydrogen peroxide initially present in the mixture was consumed after 17 hr, indicating that I had reacted with about an equimolar amount of the reagent. The yield of III at pH 7.9 after 17 hr was 73%, calculated from the ε value of III. At pH 11.0, an extensive decrease in the absorbance at 275 nm occurred within 1 min, and the formation of compounds other than non-UV-absorbing substance(s) was not significant during the reaction.

Compound III was isolated in a yield of 65% from the reaction mixture of I treated with 0.025 *M* hydrogen peroxide in 1 *M* phosphate buffer (pH 7.9) overnight. The structure of III was determined by the following observations. High-resolution mass spectroscopy and elemental analysis showed the formula of III to be $C_9H_{11}N_3O_5$. In the NMR spectrum of III, the anomeric proton signal appeared at δ 6.54 ppm with a coupling constant of 6 Hz; the signal at δ 5.46 ppm with the same coupling constant can be assigned to the 2′-proton by the double resonance technique. Chemical shift of the 2′-proton signal of III appeared very close to that of the 2,2′-cyclized compound, I[5], and was downfield from that of the uncyclized compound such as II[6], indicating that the 2,2′-anhydro linkage was retained in the molecule of III. One of the two vinylic protons at the 5- and 6-positions of I had disappeared, and one proton signal appeared at δ 7.00 ppm as a singlet. Thus, III was either the 5-hydroxy or the 6-hydroxy derivative of I.

Introduction of a hydroxy function into the 5-position of pyrimidine nucleosides produced a bathochromic and hypochromic shift of UV absorption; that at the 6-position produced a hypsochromic and hyperchromic influence (14–19). Furthermore, 5-hydroxypyrimidine nucleosides give a positive reaction with a ferric chloride solution but not with an Ehrlich's reagent, whereas 6-hydroxypyrimidine nucleosides give a positive reaction with an Ehrlich's reagent but not with a ferric chloride solution (14–19). Evidence that III was the 5-hydroxy derivative of I was provided by the UV absorption spectrum of III compared with that of I (Table I) and the violet color produced with a ferric chloride solution. The structure of III was thus established as 2,2′-anhydro-5-hydroxy-1-β-D-arabinofuranosylcytosine (Scheme I).

Compound III was quite stable in 0.1 *n* HCl at 80° for 1 hr, but it was degraded in alkali. When III was treated with concentrated ammonia at 100° for 5 hr, the major product (IV) was obtained. Compound IV, not isolated in a crystalline form, may be 5-hydroxy-1-β-D-arabinofuranosylcytosine because its UV absorption spectrum closely resembles that of the known 5-hydroxycytidine (14) and gives a positive reaction with ferric chloride solution. In 0.1 *N* NaOH, complete hydrolysis of III to IV required 6 hr at 22° and 30 min at 50° (Fig. 5); 50% hydrolysis was complete after 65 min at 22°. The anhydro bond of III was more stable than that of I, which was quantitatively converted into II in 0.1 *N* NaOH at 22° within 5 min (20). While the introduction of a halogeno group into the 5-position of I destabilized the anhydro bond (20), the introduction of a hydroxyl function stabilized the bond towards alkali.

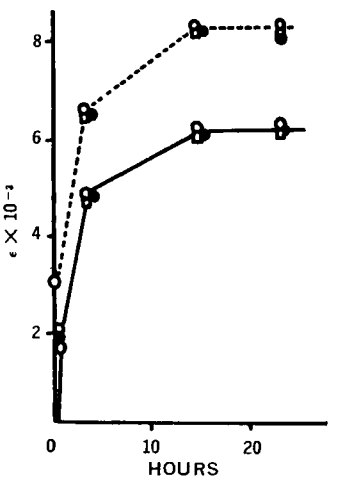
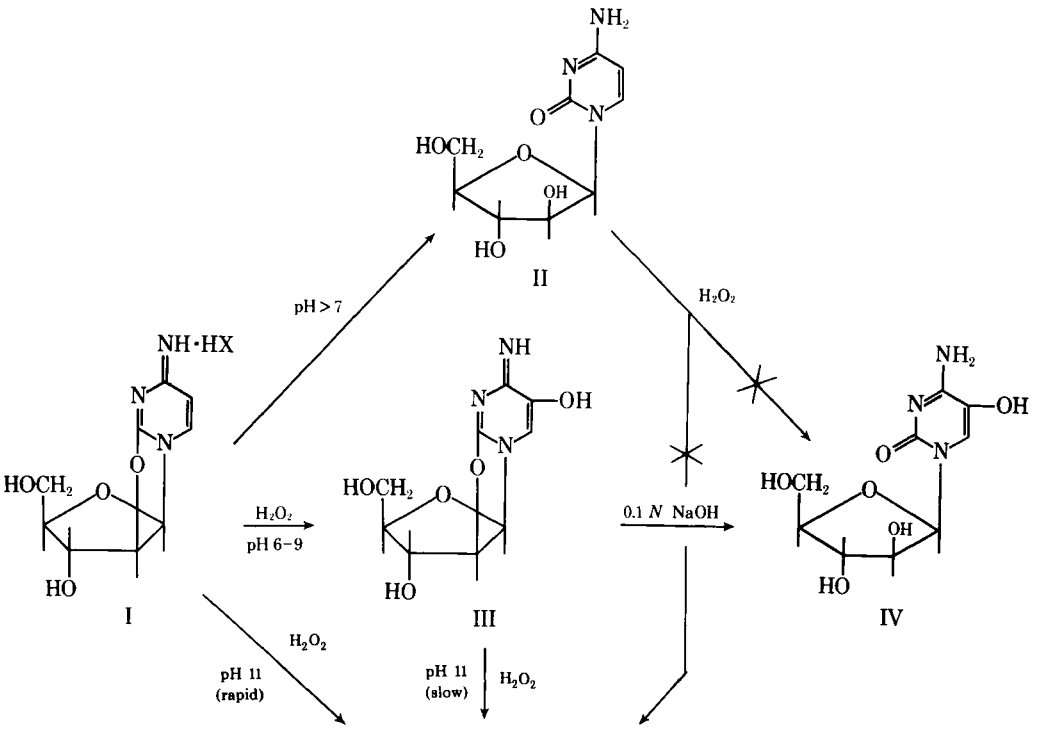


Figure 6—*Effect of ferric chloride and allyl alcohol on the reaction of I with hydrogen peroxide at pH 7.9. Compound I·HCl (7.6 × 10⁻⁴ M) was treated with 0.025 M hydrogen peroxide in 0.1 M phosphate buffer (pH 7.9) at room temperature. Absorbance at 315 nm when diluted with 0.1 N NaOH and that at 285 nm when diluted with 0.1 N HCl were measured. Key: —, 315 nm; ----, 285 nm; ○, with none; ●, with 5 × 10⁻⁵ M ferric chloride; and □, with 5 × 10⁻² M allyl alcohol.*

[5] δ 5.63 ppm (doublet, $H_{2'}$, $J_{1',2'}$ = 6 Hz).
[6] δ 4.40 ppm (triplet, $H_{2'}$, $J_{1',2'}$ = 5 Hz).



Scheme I

Formation of non-UV-absorbing substance(s) by reaction of I with hydrogen peroxide at around pH 11 was first considered to proceed *via* rapid formation and degradation of III. Thus, the reaction of III with the reagent at pH 11.0 was investigated. Although the decrease of the absorbance at 275 nm of I treated with the reagent was spontaneous and extensive (Fig. 4), that of III treated with the reagent at pH 11.0 was gradual over 20 hr, reaching about 70% of the initial one. At pH 11, the speed of transformation of III into non-UV-absorbing substance(s) was slower than that of I. Therefore, the reaction of I with hydrogen peroxide at pH 11 occurred directly without prior formation of III.

Since hydrogen peroxide is produced by redox reactions in all living cells, it may be responsible for alterations of I. The reaction of I with the reagent near neutral pH was of special interest. Reaction of I with hydrogen peroxide at pH 7.9 was not influenced by a metal ion, such as ferric chloride, or a radical trap, such as allyl alcohol, over 24 hr, indicating that the reaction of I with the reagent to form III was not due to a radical reaction (Fig. 6). In addition to II, other pyrimidine nucleosides such as cytidine, uridine, and 2,2′-anhydro-1-β-D-arabinofuranosyluracil did not suffer any significant reaction with the reagent at pH 7.9. Thus, hydroxylation of the 5-position of I with hydrogen peroxide was a reaction characteristic to the structure of I.

The effects of hydrogen peroxide on nucleic acids and their components have been investigated (21–24). The reagent with metal ions destroys the deoxyribonucleic acid molecule, causing an extensive decrease in UV absorption and melting temperature (21, 24) and liberating all four bases (21, 22). Some of the pyrimidine bases and the purine bases have been converted into non-UV-absorbing substances (22) and 2-, 7-, or 8-hydroxyadenine in the case of adenine base (23). These effects of hydrogen peroxide on nucleic acids and their components have been generally regarded as the results of radical-forming processes. The results obtained by Subbaraman *et al.* (25) on the reaction of uracil and thymine bases with alkaline hydrogen peroxide were accounted for by a mechanism in which the neutral substrate was attacked by the anion of hydrogen peroxide to form products that led to ring cleavage. The reaction of I with the reagent described here under the neutral and slightly basic conditions (pH 6–9) may be due to other mechanisms than radical-forming processes.

Compound III was studied by Hoshi *et al.* (26) as an antineoplastic agent and was found to be inactive.

SUMMARY

1. The reaction of I with a 3.4 molar excess of hydrogen peroxide (0.025 *M*) at pH 7.9 and at room temperature for 17 hr afforded III in a good yield.

2. The formation of III from I by reaction with the reagent occurred over the 6–9 pH range.

3. The reaction of I with the reagent under the neutral conditions could not be interpreted by radical-forming processes.

4. The anhydro bond of III was somewhat more stable toward alkali than that of I.

5. An extensive loss of UV absorption was observed within 1 min in the reaction of I with the reagent at pH 11, which did not proceed through II or III.

6. Compound II did not suffer significant reaction with the reagent over the 4–11 pH range.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 5, 1974, from the *Research Laboratories, Kohjin Company, Ltd., 51-Komiya-cho, Hachiouji City, Tokyo, Japan.*

Accepted for publication September 13, 1974.

Thanks are due to Mr. Motonobu Ichino of the Laboratories for the opportunity to carry out the research and for valuable information. Thanks are also due to Mr. T. Kawashima of the Laboratories and to Mr. N. Wasada of the National Chemical Laboratory for Industry for measurements of physicochemical data.

Quantitation of Propoxyphene and Its Major Metabolites in Heroin Addict Plasma after Large Dose Administration of Propoxyphene Napsylate

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Abstract □ A sensitive and specific GLC assay method was developed for the determination of propoxyphene, its major metabolite norpropoxyphene, and lesser known metabolites cyclic dinorpropoxyphene and/or dinorpropoxyphene in plasma of heroin addicts administered up to 800 mg of propoxyphene napsylate. The assay used a mass internal standard of pyrroliphene. The compounds were extracted from pH 9.8 carbonate-buffered plasma with butyl chloride, back-extracted into acidified water which was then washed with hexane, and reextracted with chloroform from the aqueous phase made basic. Quantitation of the drug and its metabolites was accomplished by temperature-programmed GLC. Absolute identification of the compounds chromatographed was completed by GLC–mass spectrometry.

Keyphrases □ Propoxyphene and metabolites—GLC analysis in heroin addict plasma □ Norpropoxyphene and cyclic dinorpropoxyphene—GLC analysis in heroin addict plasma after propoxyphene napsylate administration □ Dinorpropoxyphene (cyclic) and norpropoxyphene—GLC analysis in heroin addict plasma after propoxyphene napsylate administration □ GLC–mass spectrometry—analysis, propoxyphene and metabolites in heroin addict plasma

Propoxyphene hydrochloride[1] has been prescribed widely for the symptomatic relief of mild or moderate pain. Metabolic studies were conducted using radiocarbon-labeled propoxyphene (1) and with deuterium-labeled drug and GLC–mass spectrometry (2).

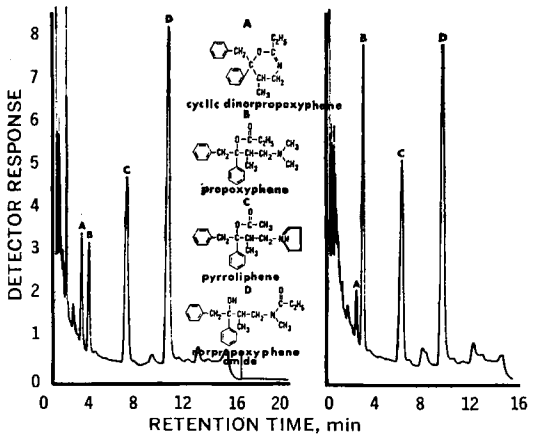

Figure 1—*Gas-liquid chromatogram (flame-ionization detector) of human plasma extracts. Left: blank plasma spiked with: Compound A, 100 ng/ml; Compound B, 100 ng/ml; Compound C, 250 ng/ml; and Compound D, 500 ng/ml. Right: patient's plasma after dose of 200 mg propoxyphene napsylate.*

The 2-naphthalenesulfonate[2], another salt of propoxyphene, has been marketed as a therapeutic agent equally as effective as the hydrochloride (3–6). The use of propoxyphene napsylate in large doses for the treatment of heroin addicts has been reported (7, 8), as has a GLC method for the determination of propoxyphene in plasma (9).

[1] α-*d*-4-(Dimethylamino)-3-methyl-1,2-diphenyl-2-propionoxybutane hydrochloride; Darvon, Eli Lilly and Co.

[2] α-*d*-4-(Dimethylamino)-3-methyl-1,2-diphenyl-2-propionoxybutane 2-naphthalenesulfonate; Darvon-N, Eli Lilly and Co.