The acetate was prepared in 58% yield using the method already described above. It was necessary, however, to extract the neutralized aqueous solution with ethyl acetate to obtain the product, which recrystallized readily from ethyl acetate–heptane (1:1) as colorless needles, m.p. 164.5–165°.[21]

The acetate was *hydrolyzed* in methanol (5 cc.) with 15 cc. of 0.047 *N* sodium hydroxide at room temperature. After one day the solution was concentrated by a cold air current, the product collected (140 mg.), recrystallized from methanol–water, and then from ethyl acetate (water) to give colorless needles, m.p. indefinite but near 122–126°. After drying at room temperature for three weeks in an evacuated desiccator this same sample melted at 167–167.5° with prior shrinking at 120°. A sample for infrared analysis was dried further in a vacuum at 110° over phosphorus pentoxide for 4 hr., m.p. 167–167.5°.

Infrared Spectra.—Percentage transmission curves were plotted from sample and solvents tracings obtained by Mr. Carl Whiteman with a Perkin–Elmer single beam recording spectrometer (Model-12A) using a rock salt prism and a 0.025 mm. thick cell. The samples were suspended in Nujol.

(21) K. Bursian (ref. 10) reports m.p. 160°.

ROCHESTER, N. Y.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF SCHERING CORPORATION]

X-Ray Diagnostics. VII. Cholecystographic Agents[1]

BY DOMENICK PAPA, HELEN F. GINSBERG, ILSE LEDERMAN AND VIRGINIA DECAMP

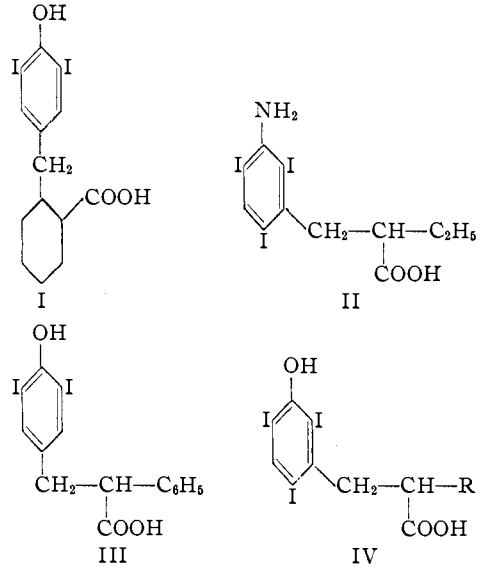
RECEIVED OCTOBER 3, 1952

The preparation of a series of α-alkyl-, α-phenyl- and α-cyclohexyl-β-(polyiodo-*m*-hydroxyphenyl)-propionic acids is described. These compounds have been obtained from *m*-hydroxy- or *m*-nitrobenzaldehyde by previously described series of transformations. Included in this study are the α-ethyl-β-(2,4,6-triiodophenyl)- and α-ethyl-β-(2,3,4,6-tetraiodophenyl)-propionic acids.

Within the last ten years, numerous studies[2] have appeared on the correlation of chemical structure and cholecystographic property. These studies have had as their objective the preparation of a cholecystographic medium combining: (a) complete absorption of the medium from the intestinal tract (no residual dye in colon); (b) high concentration of the medium in the gall bladder (optimal density); and (c) minimal or no systemic toxicity. Although the chemical and patent literature indicates that a relatively large number of compounds fulfill these requirements, only two compounds, I and II,[3] have been introduced into clinical practice since the advent of iodoalphionic acid, α-phenyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid (III).[4]

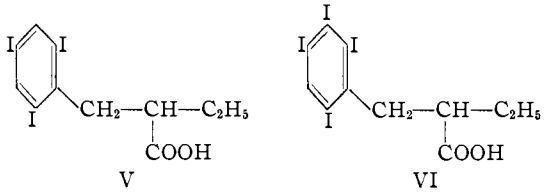
Compounds I and III have in common the diiodohydroxyphenyl group characteristic of many "cholecystographic agents," whereas compound II, embracing the triiodoaminophenyl moiety, represents a departure in this type of X-ray medium. Notwithstanding these differences, the three compounds qualitatively are equivalent in their clinical application, although they differ in degree of density of shadow, extent of systemic toxicity and quantity of residual dye in the colon.

The present study describes the preparation and preliminary pharmacological evaluation of a new series of iodinated compounds of general formula



I II III IV

IV, as well as substances V and VI. It is to be noted that compounds of general formula IV are



V VI

the hydroxy analogs of the substance of formula II, whereas compounds V and VI have neither a hydroxyl nor an amino group and, in this respect, are similar to several substances previously described,[5] for which no pharmacological data have been reported.

The compounds of general formula IV were prepared as shown in the equation

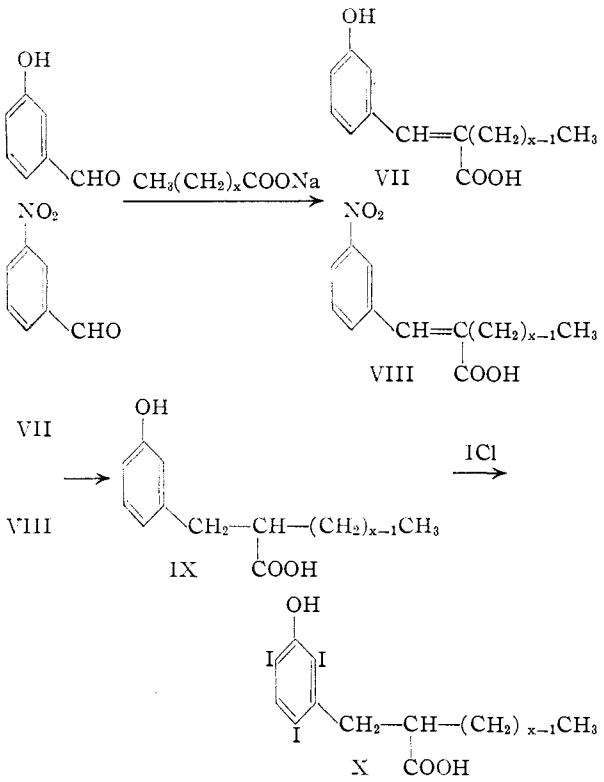
(1) Presented in abstract before the Division of Medicinal Chemistry, American Chemical Society Meeting, Atlantic City, N. J., September 15, 1952.

(2) (a) S. Natelson, B. Kramer and R. Tekel, U. S. Patent 2,400,433 (May 14, 1946); (b) E. Schwenk and D. Papa, U. S. Patent 2,436,270 (February 17, 1948); (c) B. S. Epstein, S. Natelson and B. Kramer, *Am. J. Roentgenol.*, **56**, 201 (1946); (d) D. Papa, *et al.*, THIS JOURNAL, **72**, 2619, 2623, 4906, 4909 (1950); **73**, 253 (1951); (e) S. Archer, *et al.*, *ibid.*, **71**, 3749, 3753 (1949); *J. Am. Pharm. Assoc. Sci. Ed.*, **40**, 143, 617 (1951) and others.

(3) Compound I is "Monophen" of National Synthetics (S. Natelson, B. Kramer and R. Tekel, U. S. Patent 2,496,064 (January 31, 1950)) and II is "Telepaque" of Winthrop Stearns.[2c]

(4) M. Dohrn and P. Diedrich, U. S. Patent 2,345,384 (March 28, 1944).

(5) J. W. Barnett, F. A. Robinson and B. M. Wilson, *J. Chem. Soc.*, 202 (1947), and E. Schwenk and D. Papa, U. S. Patent 2,436,270 (February 17, 1952).



The substituted cinnamic acids VII and VIII were prepared in the conventional manner using the anhydrous sodium salt of the appropriate aliphatic acid and the corresponding anhydride, except in the case of the α-phenyl and α-cyclohexyl compounds. The two latter substances were prepared by the use of potassium acetate and triethylamine, respectively, as condensing agents with the free acids. The yield of the Perkin condensation products was far superior with the nitroaldehyde than with the hydroxyaldehyde, the yields in the latter case being 56% for the α-methyl and 16% for the α-*n*-butyl compound. With the nitroaldehyde, an 80% yield of the α-ethyl compound was obtained, this yield being comparable to that previously reported.[2e]

The reduction of the cinnamic acids VII was carried out with Raney nickel catalyst in dilute alkaline solution at approximately 50 lb. pressure. The hydrogenation proceeded rapidly, the substituted propionic acids being obtained as viscous oils which crystallized on long standing. Attempts to recrystallize these compounds were generally unsuccessful and resulted in unusually large losses of product. The nitrocinnamic acids VIII were hydrogenated with Raney alloy in aqueous alkali or Raney nickel catalyst in alkaline solution under pressure. The nitropropionic acids, without isolation, were converted to the hydroxy compounds by acidifying the alkaline solution with sulfuric acid, diazotizing with sodium nitrite and heating the diazonium salt to boiling.

Iodination of the propionic acids IX was best accomplished in acetic acid solution with iodine monochloride. As the size of the alkyl group increased, the yield of the triiodo acids X fell markedly; and, in the case of the α-phenyl and α-cyclohexyl compounds, only the diiodo acids were obtained, the results paralleling those previously reported for the corresponding *m*-amino compounds.[2e] In the preliminary iodination studies with the α-ethyl compound, mixtures of diiodo and triiodo acids were obtained; but, as the iodination procedure was perfected, only triiodo acids were obtained with the α-alkyl compounds.

The synthesis of compounds V and VI was of particular interest, since no pharmacological and/or clinical data is available for substances of this type. Whether the hydroxyl or amino groups are essential for cholecystographic properties has not been established. The literature comments that the only function which these two groups serve is to facilitate the introduction of iodine; and, by inference, indicates that they do not in any way alter the cholecystographic activity of this class of radiopaque agents. Compounds V and VI were prepared from the readily available α-ethyl-β-(3-amino-2,4,6-triiodophenyl)-propionic acid by diazotization of the amino group and subsequent replacement of the diazo group by hydrogen (V) and by iodine (VI).

The pharmacological examination of the substances described in this paper indicates that the α-ethyl and α-propyl compounds of general formula IV are outstanding in the quality of gall bladder contrast and complete absence of side effects and residual medium in the colon. An extensive pharmacological comparison in dogs of the α-ethyl compound, using compounds II and III as controls, substantiates the marked superiority of the α-ethyl compound.[6] The remaining compounds of general formula IV as well as the α-phenyl and α-cyclohexyl compounds, while producing good gall bladder visualization, do not compare favorably with the α-ethyl or α-propyl compounds. Compounds V and VI were indeed disappointing in the quality of the gall bladder shadow, indicating that at least in this series of compounds the amino and hydroxyl groups markedly alter the absorption and/or excretion of the compounds and thus have a direct influence on the efficacy of these compounds as cholecystographic agents.

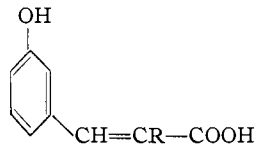
Experimental

Condensations.—The substituted cinnamic acids (Table I) were prepared as follows: α-Methyl-*m*-hydroxycinnamic acids: A mixture of 192 g. (2 moles) of anhydrous sodium propionate, 244 g. (2 moles) of *m*-hydroxybenzaldehyde and 780 g. (6 moles) of propionic anhydride was heated, with stirring, at 135–140° for seven hours. The excess anhydride was removed under vacuum, the residue dissolved in dilute alkali and heated with stirring for four to six hours. The hot alkaline solution was treated with charcoal, filtered and acidified to give 274 g. (80%) of the crude cinnamic acid melting at 132–136°. Recrystallization from water afforded 200 g. (56%) of the pure acid. Similarly the α-ethyl and α-propyl acids were obtained from sodium butyrate–butyric anhydride and sodium valerate–valeric anhydride, respectively.

The α,Δ¹-cyclohexenyl-*m*-hydroxycinnamic acid was prepared by heating, with stirring, for ten hours at 100–105°, a mixture of 280 g. (2 moles) of Δ¹-cyclohexenylacetic acid, 244 g. (2 moles) of *m*-hydroxybenzaldehyde, 202 g. (2 moles) of anhydrous triethylamine and 1,020 g. (10 moles) of acetic anhydride. The substituted cinnamic acid was isolated as

(6) S. Margolin, I. R. Stephens, M. T. Spoerlein and A. Makovsky, to be published.

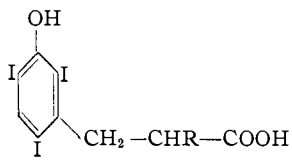
TABLE I

COMPOUNDS OF FORMULA 

R	Yield,[a] %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
CH_3	56	141–142.5[c]	$C_{10}H_{10}O_3$	67.41	67.19	5.66	5.94
C_2H_5	46	139–140	$C_{11}H_{12}O_3$	68.71	68.57	6.29	6.28
n-C_3H_7	42	136–136.5	$C_{12}H_{14}O_3$	69.88	69.31	6.84	7.07
n-C_4H_9	16	125–126	$C_{13}H_{16}O_3$	70.86	70.92	7.32	7.70
C_6H_9[b]	29	136–137	$C_{15}H_{16}O_3$	73.74	74.03	6.60	7.03
C_6H_5	60	188.5–189.5[d]	$C_{15}H_{12}O_3$	74.98	75.07	5.04	5.39

[a] The yields reported are for recrystallized compounds and represent single experimental runs. All the products were recrystallized from water or alcohol–water. [b] C_6H_9 = Δ^1-cyclohexenyl. [c] Reported m.p. 130°; *Ber.*, **28,** 2000 (1895). [d] Reported m.p. 172–173°; *Ber.*, **37,** 4132 (1904).

TABLE II

COMPOUNDS OF FORMULA 

R	Yield,[a] %	M.p., °C.	Recrystallization solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
H	80	225–226	Acetone–water	$C_9H_7O_3I_3$	19.87	19.74	1.30	1.47
CH_3	63	174–175	Acetone–water	$C_{10}H_9O_3I_3$	21.53	22.00	1.63	1.47
C_2H_5	50	142–143	Benzene–pet. ether	$C_{11}H_{11}O_3I_3$	23.13	22.97	1.94	2.00
n-C_3H_7	37	159–160	Acetone–water	$C_{12}H_{13}O_3I_3$	24.59	24.57	2.23	2.51
n-C_4H_9	19	164.5–166	Isopropyl alc.–water	$C_{13}H_{15}O_3I_3$	26.02	26.89	2.52	2.85

[a] The yields reported are for recrystallized products and represent single experimental runs.

described for the α-methyl compound and recrystallized from alcohol–water.

For the preparation of α-phenyl-*m*-hydroxycinnamic acid, a mixture of 136 g. (1 mole) of phenylacetic acid, 122 g. (1 mole) of *m*-hydroxybenzaldehyde, 98 g. (1 mole) of freshly fused potassium acetate and 408 g. (4 moles) of acetic anhydride was heated, with stirring, at 140° for 4.5 hours. After cooling, the reaction mixture was poured into ice–hydrochloric acid mixture and the oil which subsequently solidified was separated from the supernatant liquid. The crude solid acid was treated with alkali and the α-phenyl compound isolated as described for the α-methyl acid.

Hydrogenations.—The alkyl propionic acids were obtained from the α-alkyl cinnamic acids by reduction in 10% sodium hydroxide solution with Raney nickel catalyst at room temperature and 2–3 atmospheres hydrogen pressure in a conventional Parr apparatus. After 1 mole of hydrogen was absorbed, the catalyst was filtered off and the filtrate acidified with hydrochloric acid. The reduced acid was extracted with ether, the ether extracts washed with salt solution, dried over sodium sulfate and evaporated to dryness. The propionic acids were obtained as viscous oils which solidified slowly but could not be recrystallized without unusually large losses of product. In most cases the viscous oils were used directly for the iodinations.

The α,Δ^1-cyclohexenyl- and α-phenyl-*m*-hydroxycinnamic acids were reduced with Raney nickel–aluminum alloy in aqueous alkali, the α-cyclohexyl and α-phenylpropionic acids also being obtained as viscous oils. Iodinations were carried out as described for the α-alkyl compounds.

Iodination.—The preparation of α-ethyl-β-(2,4,6-triiodo-3-hydroxyphenyl)-propionic acid illustrates the iodination procedure for the α-alkyl compounds of Table II. To a stirred solution of 9.7 g. (0.05 mole) of α-ethyl-β-(*m*-hydroxyphenyl)-propionic acid in 115 cc. of glacial acetic acid, there was added, over a period of 1–1.25 hours, a solution of 29 g. of iodine monochloride in 40 cc. of acetic acid. Stirring was continued for an additional 15 minutes and then 200 cc. of water added dropwise. When almost all of the water had been added, the triiodo acid began to precipitate and during the next hour continued to form. The reaction mixture was slowly heated to 80°, kept at that temperature for a total of 40 minutes and then allowed to cool. After the excess iodine monochloride was destroyed by the addition of solid sodium bisulfite, the iodinated acid was filtered, washed with water and recrystallized from benzene–petroleum ether.

Nitroaldehyde Procedure.—The alternate procedure for the preparation of α-substituted β-(*m*-hydroxyphenyl)-propionic acids is as follows: Fifty grams of α-ethyl-*m*-nitrocinnamic acid in 10% sodium hydroxide solution was reduced with 60 g. of Raney nickel–aluminum alloy. The alkaline filtrate from the reduction was added to a mixture of 413 cc. of concentrated sulfuric acid and 666 cc. of water. After cooling to 0–5°, the amine solution was diazotized with 10 g. of sodium nitrite in 20 cc. of water. The reaction mixture was stirred an additional 15 minutes, the excess sodium nitrite destroyed with urea and the mixture heated to boiling as rapidly as possible. When the evolution of nitrogen ceased, the solution was cooled, extracted with ether, the extract dried over sodium sulfate and evaporated. The residue (35 g.), m.p. 63–66°, on iodination yielded the α-ethyltriiodo acid identical with that obtained by the *m*-hydroxybenzaldehyde procedure.

α-Phenyl-β-(2,4-diiodo-5-hydroxyphenyl)-propionic Acid.—To a stirred solution of 24 g. of α-phenyl-β-(*m*-hydroxyphenyl)-propionic acid, 20 g. of sodium hydroxide and 800 cc. of water, there was added slowly a solution of 76.2 g. of iodine and 76.2 g. of potassium iodide in 375 cc. of water. After about two-thirds of the potassium triiodide solution had been added, the rate of iodine uptake fell markedly. The remainder of the iodine solution was then added very slowly. An additional 4 g. of sodium hydroxide in 160 cc. of water was added and stirring continued until a test for free iodine was negative. The mixture was cooled to 0° with crushed ice, and acidified to litmus with solid sodium bisulfite. The crude iodinated product was purified by solution in hot sodium bicarbonate solution, the solution treated with Norite, filtered and acidified with hydrochloric acid; yield 36 g.; recrystallized for analysis from carbon tetrachloride, m.p. 185–186°.

Anal. Calcd. for $C_{15}H_{12}O_3I_2$: C, 36.46; H, 2.43. Found: C, 35.99; H, 2.50.

α-Ethyl-β-(2,4-diiodo-5-hydroxyphenyl)-propionic Acid.—Four-tenths of a mole of α-ethyl-β-(*m*-hydroxyphenyl)-propionic acid was iodinated with potassium triiodide as described for the α-phenyl compound. The gummy iodinated product from the bisulfite acidification was purified by precipitating the disodium salt from a sodium hydroxide solution with sodium chloride. The disodium salt, after solution in water and acidification, yielded a mixture of a yellow solid and an intractable oil. The solid was recrystallized from carbon tetrachloride and gave 21 g. of the diiodo acid, m.p. 115–116°.

Anal. Calcd. for $C_{11}H_{12}O_3I_2$: C, 29.14; H, 2.99. Found: C, 29.22; H, 2.71.

α-Ethyl-β-(2,4,6-triiodophenyl)-propionic Acid.—To a vigorously agitated solution of 5.7 g. of α-ethyl-β-(3-amino-2,4,6-triiodophenyl)-propionic acid in 50 cc. of concentrated sulfuric acid cooled to 0°, there was added 0.75 g. of finely powdered sodium nitrite. After an additional two hours at 0°, the reaction mixture was poured on approximately 100 g. of ice, the temperature being kept below 5°. The bright yellow slurry which formed was gradually added to a cooled, vigorously stirred suspension of 2.8 g. of cuprous oxide in 210 cc. of 95% ethanol. When the initial evolution of nitrogen had subsided, the mixture was refluxed for about 0.5 hour, at which time no further nitrogen was evolved. The ethanol suspension was diluted with an equal volume of water, kept at room temperature overnight and filtered. The triiodo compound was isolated by ether extraction of the precipitate, the ethereal solution washed with sodium thiosulfate solution, water, dried and evaporated, yield 5 g. Recrystallized from benzene–hexane for analysis, m.p. 151–152.5°.

Anal. Calcd. for $C_{11}H_{11}O_2I_3$: I, 68.5. Found: I, 68.7.

α-Ethyl-β-(2,3,4,6-tetraiodophenyl)-propionic Acid.—α-Ethyl-β-(2,4,6-triiodo-3-aminophenyl)-propionic acid (5.7 g.) in 30 cc. of concentrated sulfuric acid was diazotized with 2.1 g. of sodium nitrite. A solution of 12.7 g. of potassium iodide in 28 cc. of water was added to the cold aqueous yellow slurry of the diazonium salt; and, after the initial vigorous reaction had subsided, the mixture was heated on the steam-bath for one hour. It was then poured into a cold sodium bisulfite solution and the crude tetraiodo acid filtered; yield 7 g., m.p. 150–153°; recrystallized for analysis from acetone–water, m.p. 164–165°.

Anal. Calcd. for $C_{11}H_{10}O_2I_4$: I, 74.5. Found: I, 74.8.

Acknowledgment.—We wish to thank Dr. S. Margolin of our Pharmacology Laboratory for the animal data on the compounds and Mr. E. Conner of our Microanalytical Laboratory for the analyses reported herein.

BLOOMFIELD, NEW JERSEY

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Studies on the Structure of Nucleic Acids. VI. The Kinetics of Desoxyribonuclease Action[1]

BY LIEBE F. CAVALIERI AND BARBARA HATCH

RECEIVED OCTOBER 2, 1952

The kinetics of desoxyribonuclease action have been investigated by measuring the liberation of hydrogen ions. This was accomplished by observing the decrease in extinction of the *p*-nitrophenol–phenolate buffer system used. A plot of initial rate *vs.* initial substrate concentration exhibits a maximum which is attributed to inhibition by substrate. Since the order of reaction with respect to time is greater than that with respect to concentration, inhibition by the products of reaction is indicated. This was demonstrated to be the case experimentally. Since the inhibition by products is pronounced, it is suggested that the first products of reaction are nucleic acid-like in nature, rather than small entities. An analysis is set forth which suggests that both the inhibition by substrate and that by products involves the doubly charged phosphate anion though structural features must also be considered.

A study of the kinetics of desoxyribonuclease (DNAase) action is hampered by the fact that the products of reaction are complex entities and do not readily lend themselves to analytical procedures. Changes in viscosity, ultraviolet absorption spectrum and acid precipitability[2] are useful but inadequate since the nature of the linkages involved in these changes is not clearly understood. The measurement of the hydrogen ions produced,[2] which may be used to calculate the number of sugar–phosphate bonds cleaved, appeared to us to be the most direct and feasible route, and one which might ultimately be susceptible to interpretation in terms of the various types of bonds. In the present paper, we describe a simple colorimetric technique which may be used to measure the liberation of acid at a sensibly constant *p*H.

Experimental

Materials.—Sodium desoxyribonucleate was isolated from calf thymus, according to the procedure of Schwander and Signer[3]; $E^{1\%}_{1\,cm.}$ (water), 197. *Anal.* N, 12.7; P, 8.3. Crystalline beef pancreatic desoxyribonuclease was purchased from the Worthington Biochemical Laboratory and was used without further purification. Laboratory distilled water was redistilled from an all-glass apparatus.

Method.—The extent of DNAase action was determined by measuring the quantity of acid liberated. This was achieved by observing the change in optical density of a *p*-nitrophenol–phenolate buffer system containing the enzyme, substrate and magnesium sulfate. To relate optical density with the amount of acid produced, a standard curve was constructed by adding known increments of hydrochloric acid to the buffer system. Under the conditions of the experiments a decrease of one optical density unit at 440 mμ was brought about by the addition of 6.8×10^{-4} equivalent of acid per liter. The solvent cell contained all components except the enzyme. During enzymatic hydrolysis, the system changes by virtue of the fact that the concentration of DNA decreases. To show that this decrease in substrate concentration did not alter the standard curve, various standard curves were constructed in which the DNA was varied down to zero concentration. All curves were found to be identical.

The initial concentration of *p*-nitrophenol was 1×10^{-3} *M* in all cases. The *p*H of the reaction mixture was about 7.1. Since the pK_a of *p*-nitrophenol is 7.16, the phenol was approximately 50% neutralized and therefore at maximum buffer capacity. In general, the initial rates were calculated using values for the concentrations of DNA corre-

(1) This investigation was supported by grants from the National Cancer Institute, National Institutes of Health, United States Public Health Service, and from the Atomic Energy Commission, Contract AT(30-1)-910.

(2) M. Kunitz, *J. Gen. Physiol.*, **33**, 349 (1949).

(3) H. Schwander and R. Signer, *Helv. Chim. Acta*, **33**, 1521 (1950).