tracted with 200 ml. of isopropyl ether. The ether layer was washed with 200 ml. of water five times and dried with magnesium sulfate. The ether was removed and the residue stripped to 125° (2 mm.). The crude triester, 76 g., was obtained in 51% yield. Titration of a sample of the triester with standard alkali showed the product to be neutral.

The sodium methoxide–methanol cleavage reaction as outlined under I gave methyl *n*-octyl sulfide, 15 g. (38% theory), b.p. 85–88° (10 mm.), $n^{20}D$ 1.4580.

Anal. Calcd. for $C_9H_{20}S$: S, 20.00. Found: S, 19.85. Sulfilimine derivative, m.p. 89–90°.

VIII. O,O-Di-(2-ethylhexyl)-S-(2-octyl) Phosphorodithioate.—The 1-octene (392 g., 3.5 moles) was added dropwise to the crude O,O-di-(2-ethylhexyl) hydrogen phosphorodithioate[7] (1060 g., 3.0 moles) at such a rate that the temperature did not rise above 100°. After the addition was completed, the stirred solution was heated to 115–120° and maintained at this temperature until the acidity had decreased to a substantially constant value. The reaction mixture was cooled and washed with 10% sodium hydroxide solution. The organic layer was washed with water (sodium chloride was used to break the emulsion). The product was dried with magnesium sulfate and the excess olefin was removed by heating to a pot temperature of 125° (0.1 mm.). The residue, 1380 g., corresponds to a 95% yield of the triester. Titration with standard alkali showed the product to be neutral.

Anal. Calcd. for $C_{24}H_{51}O_2PS_2$: P, 6.66; S, 13.70. Found: P, 6.36; S, 14.04.

n-Propyl 2-Octyl Sulfide.—The cleavage of the crude O,O-di-(2-ethylhexyl)-S-(2-octyl) phosphorodithioate with sodium *n*-propoxide and *n*-propanol was carried out as outlined under I. The *n*-propyl 2-octyl sulfide was obtained in a 47% yield, b.p. 111° (15 mm.), $n^{20}D$ 1.4573.

Anal. Calcd. for $C_{11}H_{26}S$: S, 17.05. Found: S, 17.05.

The infrared patterns of this sulfide and the synthetic sample were identical.

A small amount of 2-ethylhexanol was isolated from this reaction. This was probably formed during the prolonged period of steam distillation from the alkaline medium.

The residue after steam distillation contained two layers. The organic layer was dried by refluxing with benzene and trapping out the water. The benzene was evaporated and the residue, 55 g. (53%) of sodium O,O-di-(2-ethylhexyl) phosphorothioate, was isolated. No purification of this product was carried out.

IX. O,O-Diethyl-S-(*t*-butyl) Phosphorodithioate.—The crude O,O-diethyl hydrogen phosphorodithioate, 1028 g. (5.5 moles), was placed in the stainless steel liner of a 1-gallon Aminco shaker autoclave. The liner was cooled to −75° and 355 g. (6.3 moles) of liquefied isobutylene was added. The liner was then quickly assembled in the autoclave. The autoclave was shaken and heated to 100–115° and the reaction was maintained at this temperature for six hours. The autoclave was cooled and the excess isobutylene was vented. The crude reaction product was washed with 5% sodium hydroxide, extracted with isopropyl ether, washed with 500 ml. of water, and dried over magnesium sulfate. The solvent was removed and the product fractionated *in vacuo* under nitrogen. O,O-Diethyl-S-(*t*-butyl) phosphorodithioate, 930 g., was obtained in a 70% yield; b.p. 78–80° (0.4 mm.), $n^{20}D$ 1.5023.

Anal. Calcd. for $C_8H_{19}PO_2S_2$: S, 26.5; P, 12.8. Found: S, 26.7; P, 12.8.

The sodium methoxide–methanol cleavage of O,O-diethyl-S-(*t*-butyl) phosphorodithioate was carried out as described under I. No methyl *t*-butyl sulfide was isolated; however, isobutylene was isolated from the Dry Ice trap and characterized as the α,β-dibromide,[19] b.p. 149–150°, $n^{20}D$ 1.5118.

From the residue after steam distillation, 74 g. (77%) of sodium O,O-diethyl phosphorothioate was obtained, m.p. 190–191°.

Miscellaneous Alkoxide Cleavage Reactions.—All alkoxide cleavage reactions reported in the tables and not described in the Experimental section were carried out as reported in procedure I.

Acknowledgment.—The authors wish to thank Drs. T. W. Mastin and G. R. Norman for their helpful suggestions and continued interest during this investigation and Mr. H. Ferber who carried out the phosphorus and sulfur determinations.

(19) W. Krestinsky, *Ber.*, **55**, 2757 (1922).

CLEVELAND, OHIO

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Oxygen Analogs of Pteroic Acid

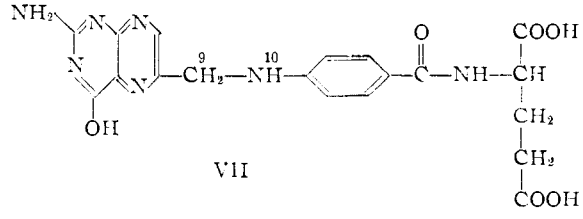
BY E. I. FAIRBURN, B. J. MAGERLEIN,[1] L. STUBBERFIELD, E. STAPERT AND D. I. WEISBLAT

RECEIVED AUGUST 31, 1953

The synthesis of four oxygen analogs of pteroic acid and pteroylglutamic acid in which the N^{10} nitrogen is replaced by oxygen is described. Data are presented showing that these compounds are folic acid antagonists.

Various analogs of pteroylglutamic acid have been reported in the literature as folic acid antagonists.[2] The chemical variations in the basic pteroylglutamic acid structure (VII) have been summarized and classified by Cosulich, *et al.*[3]

The synthesis of pteroylglutamic acid recently reported from this Laboratory[4] is admirably suited to prepare pteroic acid and pteroylglutamic acid analogs in which the N^{10} nitrogen is replaced by oxygen. The syntheses of four compounds of this type are shown in Chart I.



VII

p-Acetoxybenzoyl chloride, prepared by the method of Robertson and Robinson,[5] was condensed with diethyl L-glutamate to give diethyl *p*-acetoxybenzoyl-L-glutamate (I). Transesterification of this product with ethanol gave diethyl *p*-hydroxybenzoyl-L-glutamate (IIa) in high yield.

The alkylation of II and IIa with 2,3-oxidopropionaldehyde diethyl acetal[4] in the presence of py-

(1) Send requests for reprints to this author.

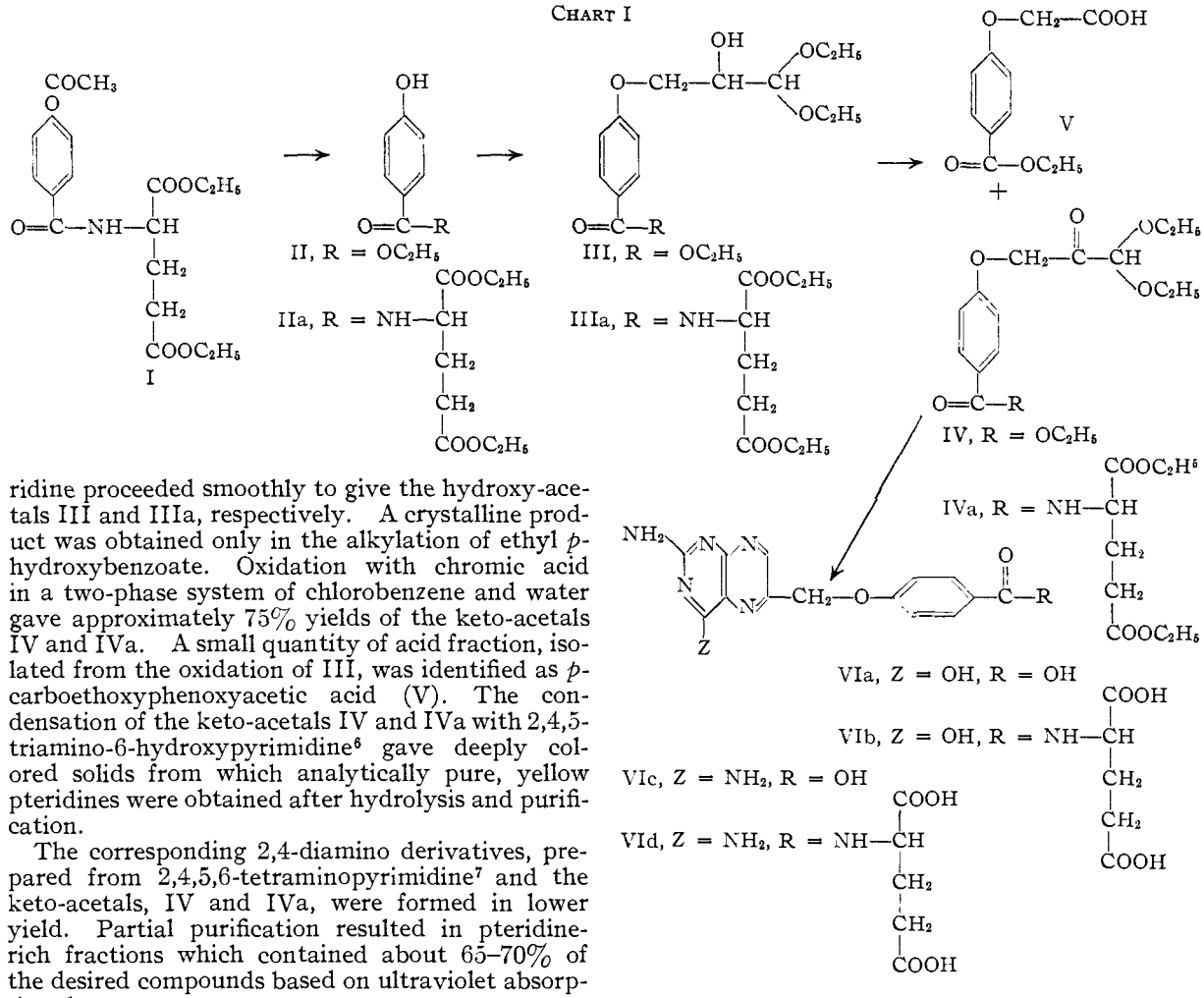
(2) J. B. Thiersch and F. S. Phillips, *Am. J. Med. Sci.*, **217**, 575 (1949); H. G. Petering, *Physiol. Rev.*, **32**, 197 (1952).

(3) D. B. Colsulich, D. R. Seeger, M. J. Fahrenbach, B. Roth, J. H. Mowat, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **73**, 2554 (1951).

(4) D. I. Weisblat, B. J. Magerlein, D. R. Myers, A. R. Hanze, E. I. Fairburn and S. T. Rolfson, *ibid.*, **75**, 5983 (1953).

(5) A. R. Robertson and R. Robinson, *J. Chem. Soc.*, 1713 (1926).

CHART I



ridine proceeded smoothly to give the hydroxy-acetals III and IIIa, respectively. A crystalline product was obtained only in the alkylation of ethyl *p*-hydroxybenzoate. Oxidation with chromic acid in a two-phase system of chlorobenzene and water gave approximately 75% yields of the keto-acetals IV and IVa. A small quantity of acid fraction, isolated from the oxidation of III, was identified as *p*-carboethoxyphenoxyacetic acid (V). The condensation of the keto-acetals IV and IVa with 2,4,5-triamino-6-hydroxypyrimidine[6] gave deeply colored solids from which analytically pure, yellow pteridines were obtained after hydrolysis and purification.

The corresponding 2,4-diamino derivatives, prepared from 2,4,5,6-tetraminopyrimidine[7] and the keto-acetals, IV and IVa, were formed in lower yield. Partial purification resulted in pteridine-rich fractions which contained about 65–70% of the desired compounds based on ultraviolet absorption data.

α-(2-Amino-4-hydroxy-6-pteridyl)-*p*-anisic acid (VIa), N-[α-(2-amino-4-hydroxy-6-pteridyl)-*p*-anisoyl]-L-glutamic acid (VIb), α-(2,4-diamino-6-pteridyl)-*p*-anisic acid (VIc) and N-[α-(2,4-diamino-6-pteridyl)-*p*-anisoyl]-L-glutamic acid (VId) possess no folic acid activity when assayed with the test microörganism *Streptococcus faecalis R.* However, they inhibit the growth of this organism in the presence of folic acid. To determine the antimetabolite activity of these analogs of pteroic acid and pteroylglutamic acid a concentration of the metabolite was dosed at a level considered to give approximately half maximal growth response for *Streptococcus faecalis R.*[8] Varying amounts of the antimetabolite were introduced and the growth of the organism was measured by titrating the acid produced after 72 hours incubation at 37°. Media and culture conditions were adapted from the procedure of Tepley and Elvehjem.[9] The biological response is calculated as the percentage of the response produced by the control level of 0.003 μg. of folic acid per tube, which represents half maximal growth under our conditions. These data are given in Table I.

TABLE I

INHIBITION STUDIES FOR PTEROIC ACID AND PTEROYLGLUTAMIC ACID ANALOGS

Inhibitor, μg.	Folic acid, μg.	Wt. ratio of inhibitor to folic acid	Bio. response, % Compd. VIa	Compd. VIb	Compd. VIc	Compd. VId
0	0.003	.....	100.0	100	100	100
0.003	.003	1–1	106.0	115	103.3	101.7
0.03	.003	10–1	103.3	83	99.2	99.2
0.3	.003	100–1	78.3	37.5	42.5	85.0
3.0	.003	1000–1	28.3	None	None	21.7
30.0	.003	10,000–1	None	...	...	None

Reversal studies were carried out using constant amounts of antimetabolite from a point which completely inhibited the growth of *Streptococcus faecalis R* to a point at which the metabolite would overcome this inhibition (Table II). No toxic effects of the antimetabolite could be detected at the levels used. The antimetabolite activity of compounds VIa, VIb and VId could be completely reversed by folic acid while the effect of VIc was not completely reversed under the conditions of the experiment.

Preliminary screening of α-(2-amino-4-hydroxy-6-pteridyl)-*p*-anisic acid (VIa) against Sarcoma 180 by Dr. C. C. Stock, of the Sloan–Kettering Insti-

(6) W. Traube, *Ber.*, **33**, 1371 (1900).

(7) W. Traube, *ibid.*, **37**, 4544 (1904); M. F. Malette, E. C. Taylor, Jr., and C. K. Cain, THIS JOURNAL, **69**, 1814 (1947).

(8) D. W. Wooley, "A Study of Antimetabolites," John Wiley and Sons, Inc., New York, N. Y., 1952, p. 66.

(9) L. J. Tepley and C. A. Elvehjem, *J. Biol. Chem.*, **157**, 303 (1945).

TABLE II

REVERSAL STUDIES FOR PTEROIC ACID AND PTEROYLGLUTAMIC ACID ANALOGS

Inhibitor, μg.	Folic acid, μg.	Wt. ratio of inhibitor to folic acid	Bio. response, % Compd. VIa	Compd. VIb	Compd. VIc	Compd. VId
0	0.003		100.0	100	100	100
3	.003	1,000–1	28.0	None	None	30.9
3	.006	500–1	69.0	15.5	None	...
3	.03	100–1	>100	>100	3.6	105.0
3	.3	10–1	>100	>100	27.7	>100
30	.003	10,000–1	None	None	...	12.5
30	.03	1,000–1	20.0	None	33.3	40.0
30	.3	100–1	>100	>100	36.7	73.4
30	1.0	33–1	>100	>100	...	...
30	3.0	10–1	...	...	38.4	>100
30	30.0	1–1	...	...	72.5	...
30	300.0	0.1–1	...	...	89.2	...

tute, and against the Walker rat carcinoma 256 and a rat lymphosarcoma by Dr. E. S. Feenstra, of these laboratories, failed to show appreciable tumor inhibition.

Acknowledgment.—The authors are indebted to Mr. O. F. Swoap and associates for the toxicity studies of these compounds; to Dr. G. Pish and Mr. L. Scholten for the ultraviolet absorption data; and to Mr. W. A. Struck and associates for the microanalyses.

Experimental[10]

Diethyl *p*-Acetoxybenzoyl-L-glutamate (I).—Eighteen grams of *p*-acetoxybenzoic acid was converted to the acid chloride using thionyl chloride in a toluene solution.[5] The solvent was distilled under vacuum and the residue dissolved in 100 ml. of ethylene dichloride. To this solution there was added 24 g. of diethyl L-glutamate hydrochloride dissolved in 90 ml. of ethylene dichloride. The combined solution was cooled to 20° and a solution of 22.2 g. of triethylamine in 60 ml. of ethylene dichloride was added at such a rate that the temperature did not exceed 20°. After stirring at 25° for one hour the reaction mixture was washed with successive portions of water, dilute hydrochloric acid, sodium bicarbonate solution and water. Evaporation of the solvent, after drying over anhydrous sodium sulfate, gave 33.1 g. of crude product. Crystallization from ether gave 20.9 g. (60%) of I, m.p. 74–76.5°. Recrystallization from 2-propanol raised the melting point to 75–76.5°.

Anal. Calcd. for $C_{18}H_{23}NO_7$: C, 59.16; H, 6.34; N, 3.83. Found: C, 59.12, 59.31; H, 6.34, 6.31; N, 3.90, 3.92.

Diethyl *p*-Hydroxybenzoyl-L-glutamate (IIa).—A solution of 10 g. of diethyl *p*-acetoxybenzoyl-L-glutamate and 3 g. of *p*-toluenesulfonic acid monohydrate in 200 ml. of absolute ethanol was heated under reflux for 40 minutes. Over a period of 5 hours about 75% of the alcohol was distilled, using a 12-inch, helix-packed column fitted with a total reflux, partial take-off distilling head. The solution was evaporated to dryness under vacuum. The residue was dissolved in ethyl acetate, washed with sodium bicarbonate solution, and dried over sodium sulfate. Distillation of the solvent gave 8.2 g. of IIa, m.p. 78–85°. Recrystallization from 2-propanol gave a sample melting 90–91°; $[\alpha]^{24}D$ −14.4° (95% ethanol).

Anal. Calcd. for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.68; H, 6.40; N, 4.23.

The Ethyl *p*-(2-Hydroxy-3,3-diethoxypropoxy)-benzoate (III).[11]—At 130°, 332 g. of ethyl *p*-hydroxybenzoate was fused and 292 g. of 2,3-oxidopropionaldehyde diethyl acetal[4] added. After a clear solution formed, 0.5 ml. of pyridine was added. The temperature of the reaction was gradually brought to 158°. This required about 15 minutes. At the end of this period an additional 0.5 ml. of pyridine was added and the reaction temperature rose rapidly to 175°. The reaction temperature was cooled to 155° and maintained at 155–160° for 90 minutes. The reaction mixture was cooled and dissolved in 500 ml. of ether. The organic solution was washed twice with 500-ml. portions of water and twice with 250-ml. portions of 3% sodium hydroxide solution. The organic layer was then washed twice more with water and dried by shaking with saturated sodium chloride solution and filtering through anhydrous sodium sulfate. The ether was distilled and the residue distilled under vacuum, b.p. 171–175° (0.6–0.65 mm.). There was obtained 460 g. (73.5%) of a yellow oil. The product crystallized on standing. Recrystallization from Skellysolve B gave white crystals, m.p. 30–33.5°.

Anal. Calcd. for $C_{16}H_{24}O_6$: C, 61.52; H, 7.74. Found: C, 62.17, 62.21; H, 7.93, 7.64.

Ethyl *p*-(2-Keto-3,3-diethoxypropoxy)-benzoate (IV).—To a precooled solution of 275 g. of sodium dichromate in 1210 ml. of water and 361 ml. of concentrated sulfuric acid was added with vigorous stirring a solution of 163 g. of ethyl *p*-(2-hydroxy-3,3-diethoxypropoxy)-benzoate (III) in 2 l. of chlorobenzene. The addition was made at such a rate that the reaction temperature could be maintained between 5–8° by cooling in an ice-bath.

At the end of one hour benzene was added and the organic layer separated. The water soluble layer was extracted with two 500-ml. portions of benzene. The organic layers were combined and washed once with 1.5 l. of water and twice with 1.5-l. portions of a solution made by mixing water and saturated sodium chloride solution in a ratio of 1:1 by volume. The organic layer was dried by shaking with saturated sodium chloride solution and stirring with anhydrous sodium sulfate. The solvent was distilled under vacuum and there was obtained 123 g. (75%) of a yellow oil. This material could not be crystallized.

Extraction of the benzene solution of the keto-acetate IV with saturated sodium bicarbonate solution followed by acidification gave the acid (V), m.p. 130–132.5°.

Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.92; H, 5.39. Found: C, 58.78, 59.02; H, 5.37, 5.15.

Diethyl *p*-(2-Hydroxy-3,3-diethoxypropoxy)-benzoyl-L-glutamate (IIIa).—A mixture of 6.46 g. of diethyl *p*-hydroxybenzoyl-L-glutamate (IIa) and 3.2 g. of 2,3-oxidopropionaldehyde diethyl acetal was fused by heating to 120°. Seven drops of pyridine were added and the temperature of the melt maintained at 135° for 2 hours. The reaction mixture was worked up similarly to the preparation of III to give 8.6 g. of oil which resisted crystallization.

Diethyl *p*-(2-Keto-3,3-diethoxypropoxy)-benzoyl-L-glutamate (IVa).—The crude IIIa from above was dissolved in 75 ml. of chlorobenzene and oxidized as described above with a solution of 10.5 g. of sodium dichromate in 46 ml. of water and 13.8 ml. of concentrated sulfuric acid. The product, which weighed 6.6 g., was a yellow oil.

α-(2-Amino-4-hydroxy-6-pteridyl)-*p*-anisic Acid (VIa).—To a mixture of 0.42 g. of sodium acetate and 0.55 g. of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride was added 0.80 g. of ethyl *p*-(2-keto-3,3-diethoxypropoxy)-benzoate dissolved in 14.8 ml. of glacial acetic acid. The mixture was stirred at 25° in the dark and under an atmosphere of nitrogen for 30 minutes. The mixture was then stirred for 20 minutes at 118–120°, cooled in an ice-bath, and centrifuged. The black residue was washed twice with water and once with acetone. This product weighed 0.58 g.

The black condensation product was dissolved in 10% sodium hydroxide. After one hour the *p*H of the solution was adjusted to 3.0. The product was recovered by centrifugation and dried to give 0.49 g. of crude VIa, $\lambda_{max}^{0.1N\ NaOH}$ 257 mμ, $E_{1\ cm.}^{1\%}$ 880; 364 mμ, $E_{1\ cm.}^{1\%}$ 224.

Of the crude saponified product, prepared as described above, 0.95 g. was dissolved in 100 ml. of *N* sodium hydroxide and diluted to 500 ml. with water. To the solution was added 0.95 g. of calcium hydroxide, and the mixture was stirred at 25° in the dark for 90 minutes and filtered. The filtrate was heated to boiling and filtered, reheated to boiling and the *p*H adjusted to 3.0 with concentrated hydrochloric acid. A yellow precipitate settled which was recovered by centrifugation and washed three times with water. The product, dried under vacuum, weighed 0.743 g., $\lambda_{max}^{0.1\ N\ NaOH}$ 257 mμ, $E_{1\ cm.}^{1\%}$ 1114; 363 mμ, $E_{1\ cm.}^{1\%}$ 267. A sample for analysis was dried at 100° for 7 hours.

Anal. Calcd. for $C_{14}H_{11}N_5O_4$: C, 53.67; H, 3.54; N,

(10) All melting points are uncorrected.

(11) The authors are indebted to Dr. W. B. Reid, Jr., of these laboratories, for this preparation.

22.35. Found: C, 54.34, 54.21; H, 3.64, 3.73; N, 22.35, 22.48; dry ash, 0.40.

N-[α-(2-Amino-4-hydroxy-6-pteridyl)-*p*-anisoyl]-L-glutamic Acid (VIb).—To a mixture of 1.38 g. of sodium acetate and 1.80 g. of 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride was added 2.7 g. of crude diethyl *p*-(2-keto-3,3-diethoxypropoxy)-benzoyl-L-glutamate dissolved in 48 ml. of glacial acetic acid. The mixture was stirred under nitrogen and in the dark for 30 minutes at 25° and for one hour at 105–110°. The reaction mixture was cooled and centrifuged. The black solid was washed three times with water, dried and then washed with ethyl acetate. There was obtained 0.2 g. of material, $\lambda_{max}^{0.1\ N\ NaOH}$ 258 mμ, $E_{1\ cm.}^{1\%}$ 643; 366 mμ, $E_{1\ cm.}^{1\%}$ 178.

The acetic acid supernatant from the centrifuged product was concentrated and the black residue washed with water and ethyl acetate to give 1.37 g. of product, $\lambda_{max}^{0.1\ N\ NaOH}$ 258 mμ, $E_{1\ cm.}^{1\%}$ 674; 364 mμ, $E_{1\ cm.}^{1\%}$ 148. The combined products weighed 1.57 g. (60.8%).

A 0.5-g. sample of the crude pteridine was dissolved in 100 ml. of 0.1 *N* sodium hydroxide and diluted with 2 l. of water. To the solution was added 0.5 g. of calcium hydroxide and the mixture was stirred for 90 minutes, then heated to boiling and filtered. The solution was reheated to boiling and the *p*H adjusted to 3.0. A brown precipitate which settled on standing was collected, washed with water, and dried. There was obtained 0.24 g. of material $\lambda_{max}^{0.1\ N\ NaOH}$ 258 mμ, $E_{1\ cm.}^{1\%}$ 855; 364 mμ, $E_{1\ cm.}^{1\%}$ 185. Recrystallization from water containing a few drops of acetic acid gave a yellow solid which was collected by centrifugation. The solid was lyophilized and then dried for 5 hours at 100°.

Anal. Calcd. for $C_{19}H_{18}N_6O_7$: C, 51.58; H, 4.09; N, 18.99. Found: C, 51.08, 50.98; H, 4.25, 4.32; N, 18.67, 18.72; $\lambda_{max}^{0.1\ N\ NaOH}$ 259 mμ, $E_{1\ cm.}^{1\%}$ 889, 364 mμ, $E_{1\ cm.}^{1\%}$ 185.

α-(2,4-Diamino-6-pteridyl)-*p*-anisic Acid (VIc).—The *p*H of a mixture of 6.36 g. of 2,4,5,6-tetraminopyrimidine bisulfite[6] in 120 ml. of 65% ethanol was adjusted to 3.0 with dilute hydrochloric acid. A solution of 8.91 g. of IV in 40 ml. of 95% ethanol was added over a period of 20 minutes. The mixture was heated at 90° for 2 hours, then 3 ml. of concentrated hydrochloric acid added and heated for 15 minutes at 100°. The *p*H of the solution was adjusted to 4.5 with sodium hydroxide and the solution cooled to 2°. The yellow precipitate was collected by centrifugation and lyophilized to give 7.39 g. of product, $\lambda_{max}^{0.1\ N\ NaOH}$ 256 mμ, $E_{1\ cm.}^{1\%}$ 463; 364 mμ, $E_{1\ cm.}^{1\%}$ 130.

Saponification of 2.0 g. of the above material with 10% sodium hydroxide at room temperature gave 1.1 g. of crude VIc, $\lambda_{max}^{0.1\ N\ NaOH}$ 256 mμ, $E_{1\ cm.}^{1\%}$ 751; 364 mμ, $E_{1\ cm.}^{1\%}$ 200. Purification of this material was not attempted.

N-[α-(2,4-Diamino-4-hydroxy-6-pteridyl)-*p*-anisoyl]-L-glutamic Acid (VId).—In the manner described above 6.28 g. of IVa was treated with 2.96 g. of 2,4,5,6-tetraminopyrimidine bisulfite to give 3.95 g. of yellow solid, $\lambda_{max}^{0.1\ N\ NaOH}$ 256 mμ, $E_{1\ cm.}^{1\%}$ 559; 264 mμ, $E_{1\ cm.}^{1\%}$ 166. Saponification with 10% sodium hydroxide gave a yellow solid $\lambda_{max}^{0.1\ N\ NaOH}$ 257 mμ, $E_{1\ cm.}^{1\%}$ 528; 364 mμ, $E_{1\ cm.}^{1\%}$ 143. A mixture of crude VId and 250 mg. of calcium hydroxide in 75 ml. of 0.5 *N* sodium hydroxide was stirred for 1.5 hours at 25° and filtered. The *p*H was adjusted to 3.0 and the precipitate which formed collected and dried (40 mg.). Refrigeration of the mother liquors gave an additional 30 mg. of partially purified VId, $\lambda_{max}^{0.1\ N\ NaOH}$ 259 mμ, $E_{1\ cm.}^{1\%}$ 729; 368 mμ, $E_{1\ cm.}^{1\%}$ 167.

KALAMAZOO, MICHIGAN

[CONTRIBUTION FROM THE MCARDLE MEMORIAL LABORATORY, THE MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN]

Intramolecular Heterogeneity in Nucleic Acid Biosynthesis[1,2]

BY KIVIE MOLDAVE[3] AND CHARLES HEIDELBERGER

RECEIVED SEPTEMBER 30, 1953

Non-uniform labeling of the nucleotide phosphorus and pyrimidines has been found in partial ribonuclease digests of RNA from cell fractions of rat liver and Flexner–Jobling carcinoma following simultaneous administration of P^{32}, orotic acid-6-C^{14} and glycine-2-C^{14}. Uniform labeling of phosphorus, pyrimidines and purines from DNA degradations, and of RNA purines has been demonstrated. Such non-uniformity was not found with alkaline hydrolysates of RNA. The specific activities of the phosphorus of a given 2′- and 3′- or 5′-mononucleotide were approximately the same. Terminal phosphorus had a higher specific activity than average nucleotide phosphorus. The compositions of partial ribonuclease digests varied among the cell fractions.

There is now at hand abundant evidence from this Laboratory and many others that demonstrates intracellular heterogeneity in nucleic acid biosynthesis. When radiophosphorus is administered to animals, the ribonucleic acid (RNA) isolated from the nuclei of liver and other tissues has a higher specific activity than that of the cytoplasmic particles; the specific activity of the RNA from the soluble fraction is intermediate.[4–7] A similar phenomenon is observed in the RNA purines following administration of glycine-2-C^{14} [6] and in the RNA pyrimidines biosynthesized from orotic acid-6-C^{14}.[8]

It was felt that further information might be gained concerning the mechanisms of nucleic acid biosynthesis in normal and neoplastic tissues by an investigation of the intramolecular distribution of radioactivity in partial degradation products of nucleic acids. We were encouraged in this belief by the interesting results of Steinberg and Anfinsen[9] who found non-uniform labeling in various fractions derived from ovalbumin biosynthesized both *in vitro* and *in vivo* in the presence of labeled carbon dioxide or alanine. Furthermore, Bendich, *et al.*,[10] have isolated from each of several rat tissues two desoxyribonucleic acids (DNA) that differ in their physical and metabolic characteristics.

(1) This work was supported in part by a grant from the Wisconsin Section of the American Cancer Society, and in part by an Institutional grant from the American Cancer Society.

(2) An abstract of part of this work appears in *Federation Proc.*, **12**, 247 (1953).

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(4) C. P. Barnum and R. A. Huseby, *Arch. Biochem.*, **29**, 7 (1950).

(5) R. Jeener and D. Szafarz, *ibid.*, **26**, 54 (1950).

(6) E. P. Tyner, C. Heidelberger and G. A. LePage, *Cancer Research*, **13** 186 (1953).

(7) J. N. Davidson and R. M. S. Smellie, *Biochem. J.*, **52**, 599 (1952).

(8) R. B. Hurlbert and V. R. Potter, *J. Biol. Chem.*, **195**, 257 (1952), and in press.

(9) D. Steinberg and C. B. Anfinsen, *ibid.*, **199**, 25 (1952).

(10) A. Bendich, P. J. Russell and G. B. Brown, *ibid.*, **203**, 305 (1953).