[CONTRIBUTION FROM THE NAGOYA INDUSTRIAL SCIENCE RESEARCH INSTITUTE,* ROKUNO-CHŌ ATSUTA-KU,* AND THE CHEMICAL INSTITUTE, NAGOYA UNIVERSITY**]

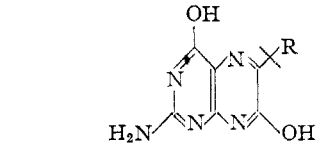
Studies on Pteridines. V. Reductive Cleavage of Pteridyl Side Chains[1]

BY SABURO NAWA,* SADAO MATSUURA* AND YOSHIMASA HIRATA**

RECEIVED MARCH 12, 1953

Certain pteridyl side chains are cleaved by treatment with Al–Hg in alkali, and the reaction has proven to be useful for characterization of pteridines.

We have previously isolated a pteridine from carp scale[2] and have shown it to belong to the isoxanthopterin series. Various pteridines have subsequently been synthesized as a means for structural elucidation[3] and it has been found that the side chains of certain pteridines are cleaved under reductive conditions and that this specific reaction may be utilized advantageously for structure determinations. Namely, those isoxanthopterin derivatives with R = –COOH (II), –CH(OH)CH_3 (III), –CH_2NH_2 (IV) and –CH_2-$COCH_3$ (V) were cleaved at the position indicated with broken lines when treated with Al–Hg in alkaline media. Thus, in addition to isoxanthopterin (I), acetone was obtained in a high yield from 6-acetonylisoxanthopterin (V), and methylamine from 6-methylaminoisoxanthopterin (IV). These reactions indicate the almost quantitative cleavage of the C–C bond between the nucleus and the side chain through this reductive treatment. Elion and Hitchings[4] have reported that the heating (150°) of the dihydro derivative of isoxanthopterin-6-carboxylic acid (II) obtained by reducing II with Zn and alkali or with sodium amalgam resulted in decarboxylation. However, we have observed that when II was reduced at room temperature and the reaction mixture was submitted to paper chromatography an almost complete conversion into isoxanthopterin had occurred. When R = –CH_3 (VI), –C_2H_5 (VII), –C_3H_7 (VIII), –CH_2COOH (IX), –$CH(CH_3)COOH$ (X), –CH_2CH_2COOH (XI), –$CH_2CH_2OCH_3$ (XII) and –$CH_2CH(OH)CH_3$ (XIII), no reaction took place.

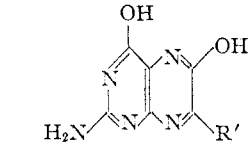


R = —H (I), —COOH (II)
—CHCH$_3$ (III)
OH
—CH_2NH_2 (IV), —CH_2COCH_3 (V)

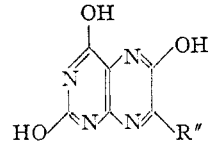
The product obtained from carp scale was cleaved to yield isoxanthopterin (I). Aluminum in alkali, Mg in boiling water or Al or Fe in dilute HCl also yielded isoxanthopterin from isoxanthopterincarboxylic acid (II).

Paper chromatography[1] was employed as a means for the identification of the pteridines; when necessary, the ultraviolet absorption spectra[1] were also compared.

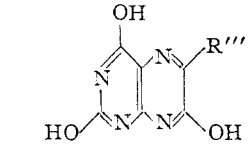
It was found that similar reaction took place in the xanthopterin series as well. Namely, xanthopterin (XIV) was obtained from xanthopterincarboxylic acid (XV) and 6-acetonylxanthopterin (XVI), respectively, when these were treated with Al–Hg; acetone was isolated from (XVI). Similar reactions were also observed with 2-hydroxypteridines XVII–XXII.



R′ = –H (XIV)
–COOH (XV)
–CH_2COCH_3 (XVI)



R″ = –H (XVII)
–COOH (XVIII)
–CH_2COCH_3 (XIX)



R‴ = –H (XX)
–COOH (XXI)
–CH_2COCH_3 (XXII)

Experimental

Isoxanthopterin and Acetone from 6-Acetonylisoxanthopterin (V).—To a solution of 100 mg. of 6-acetonylisoxanthopterin in 3 ml. of 5% NaOH, there was added 1 g. of Al–Hg and the mixture was heated on the bath for 10 minutes; the acetone was collected together with the hydrogen gas into a solution of 10 mg. of 2,4-dinitrophenylhydrazine dissolved in 5 ml. of ethanol and 0.2 ml. of sulfuric acid. After 20 minutes, the crystals were filtered, washed with ethanol and dried; yellow needles, 85 mg. (85%), m.p. 128°. The alkaline solution remaining in the original flask was filtered, acidified and the precipitate was collected. This was dissolved in 5 ml. of 3% NaOH and the solution was added dropwise to 10 ml. of boiling 3% HCl, when 60 mg. (80%) of isoxanthopterin was obtained. This was confirmed by paper chromatography and ultraviolet spectrum.

Anal. Calcd. for $C_6H_5O_2N_5$: N, 39.11. Found: N, 38.89.

Methylamine from 6-Methylaminoisoxanthopterin (IV).—To a solution of 100 mg. of IV in 10 ml. of 5% NaOH, there was added 1 g. of Al–Hg and the mixture was heated on the bath; the gas was passed into 10 ml. of an ethereal solution of 100 mg. of picric acid. The prisms were collected after 20 minutes, washed with ether and dried; 16 mg. (13%), m.p. 195°. As a control, methylamine was generated from the hydrochloride, and the picrate (198°) was obtained in 24% yield under similar conditions.

Isoxanthopterin from Isoxanthopterincarboxylic Acid (II).—One hundred mg. of (II) was treated with Al–Hg in a similar manner and the solution was acidified with HCl. The precipitate was dissolved in 5 ml. of 3% NaOH and the solution was added dropwise to 10 ml. of boiling 3% HCl; the crystals were collected, washed and dried; 71 mg. (89%).

(1) Paper IV, S. Matsuura, S. Nawa, H. Kakizawa and Y. Hirata, THIS JOURNAL, **75**, 4446 (1953).

(2) Y. Hirata and S. Nawa, *Compt. rend. soc. biol.*, **145**, 661 (1951).

(3) Y. Hirata, S. Nawa, S. Matsuura and H. Kakizawa, *Experientia*, **8**, 339 (1952).

(4) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **74**, 3878 (1952).

Anal. Calcd. for $C_6H_6O_2N_6$: N, 39.11. Found: N, 39.24.

Acetone from 7-Acetonylxanthopterin (XVI).—Under similar treatment as mentioned for 6-acetonylisoxanthopterin (V), 66 mg. (66%) of acetone 2,4-dinitrophenylhydrazone was obtained from 7-acetonylxanthopterin (XVI). The residual xanthopterin being in the reductive state had no fluorescence, but addition of MnO_2 regenerated the fluorescence and xanthopterin was identified through paper chromatography.

Miscellaneous.—Acetone was also obtained in yields of 75% and 66% from 2,4,6-trihydroxy-7-acetonylpteridine (XIX) and 2,4,7-trihydroxy-6-acetonylpteridine (XXII), respectively. Xanthopterincarboxylic acid (XV), 2,4,6-trihydroxypteridyl-7-carboxylic acid (XVIII) and 2,4,7-trihydroxypteridyl-6-carboxylic acid (XXI) were also decarboxylated under the influence of Al–Hg to give xanthopterin (XIV), 2,4,6-trihydroxypteridine (XVII) and 2,4,7-trihydroxypteridine (XX), respectively.

Acknowledgment.—The authors are greatly indebted to Prof. Fujio Egami, under whose guidance the present study has been carried out, and to Mr. Koji Nakanishi for valuable advice. A part of the cost of this research was defrayed from the Scientific Research Encouragement Grant from the Ministry of Education.

NAGOYA, JAPAN

[CONTRIBUTION FROM THE DEPARTMENT OF INDUSTRIAL MEDICINE, NEW YORK UNIVERSITY POST-GRADUATE MEDICAL SCHOOL]

The Interaction of Dinitrobenzene Derivatives with Bovine Serum Albumin[1,2]

BY MARY E. CARSTEN AND HERMAN N. EISEN

RECEIVED MARCH 27, 1953

The reversible interaction of a series of ionic and non-ionic substituted 2,4-dinitrobenzenes with bovine serum albumin has been studied at two temperatures. They differed from each other only in the substituent at carbon one. Included in the series were two 2,4-dinitrophenyl amino acids. The nature of the substituent at carbon one was found to modify considerably the binding affinity. Relative binding affinities were obtained graphically and energies of interaction (ΔH^0) computed. Chloride ion inhibits the binding of dinitrophenol by bovine serum albumin but not that of dinitroaniline. Dinitrotoluene also inhibits binding of dinitrophenol. Binding to bovine serum albumin results in shifts in the absorption spectra of ε-dinitrophenyl-aminocaproic acid, dinitroaniline and dinitrophenol.

The interactions of inorganic and organic ions with serum albumins have been studied extensively. Little attention, however, has been given until very recently to the binding of neutral organic molecules.[3] We have studied the reversible interactions of bovine serum albumin with a series of ionic and non-ionic substituted 2,4-dinitrobenzenes which differ only in the substituent at carbon one. Included in this series were some 2,4-dinitrophenyl (DNP) amino acids. The compounds studied made it possible to evaluate the contribution of the various substituents to the binding and, in particular, to obtain information on the ionic contribution to the binding. The binding of an ionic compound in the presence of a non-ionic homolog has made it possible to gain some insight into the specificity of binding sites.

Furthermore, substituted 2,4-dinitrobenzenes are of considerable interest from an immunologic viewpoint. Some of these compounds, under appropriate conditions, give rise to a wide range of allergic reactions.[4] The interaction of these compounds with serum albumin provides a basis for interpreting some of these biological phenomena[5] and is of particular interest in relation to corresponding interactions involving antibodies specific for the 2,4-dinitrophenyl group. The latter problem is under study in this Laboratory.

The present paper records measurements of the binding of the following 2,4-dinitrobenzene derivatives by bovine serum albumin: 2,4-dinitrophenol, 2,4-dinitrobromobenzene, 2,4-dinitroaniline, 2,4-dinitrotoluene, *m*-dinitrobenzene, ε-N-dinitrophenyllysine (ε-DNP-lysine) and ε-dinitrophenyl-aminocaproic acid (ε-DNP-aminocaproic acid). Competition of various compounds for the binding sites of the albumin molecule was studied in the following pairs: dinitrophenol and dinitrotoluene, dinitrophenol and chloride ion, dinitroaniline and chloride ion. The absorption spectra of dinitrophenol, dinitroaniline and ε-DNP-aminocaproic acid bound to serum albumin were obtained. Under the experimental conditions, dinitrophenol and ε-DNP-aminocaproic acid were in anionic form, while ε-DNP-lysine was a dipolar ion.

Experimental

Materials and Methods.—Crystalline bovine serum albumin, obtained from Armour and Company, was used. Protein concentrations were measured in a model DU Beckman spectrophotometer. The spectrophotometer reading was calibrated with micro-Kjeldahl nitrogen values for a bovine serum albumin solution. The protein concentration was calculated, using a value of 16.07% nitrogen[6] and a molecular weight of 69,000.[7]

Commercial samples of dinitrophenol, dinitrobromobenzene, dinitroaniline, dinitrotoluene and *m*-dinitrobenzene were twice recrystallized. ε-DNP-lysine was prepared according to the method of Porter.[8] Its purity was indicated chromatographically and by a melting point of 182° (literature 180°[8]).

ε-DNP-Aminocaproic acid was prepared from ε-aminocaproic acid according to the method given by Sanger[9] for DNP-phenylalanine. Precipitation of ε-DNP-aminocaproic acid by acidification was repeated three times to remove un-

(1) The support of this work by grants from the United States Public Health Service and from the Standard Oil Co. (N. J.), New York City, is gratefully acknowledged.

(2) A preliminary report was presented at the meeting of the American Society of Biological Chemists: *Federation Proc.*, **12**, 187 (1953).

(3) I. M. Klotz and J. Ayers, THIS JOURNAL, **74**, 6178 (1952).

(4) K. Landsteiner and M. W. Chase, *J. Exp. Med.*, **66**, 337 (1937).

(5) H. N. Eisen, L. Orris and S. Belman, *ibid.*, **95**, 473 (1952).

(6) E. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).

(7) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, THIS JOURNAL, **69**, 1753 (1947).

(8) R. R. Porter in "Methods of Medical Research," Vol. 3, The Year Book Publishers, Inc., Chicago, Ill., 1950, p. 256.

(9) F. Sanger, *Biochem. J.*, **39**, 507 (1945).