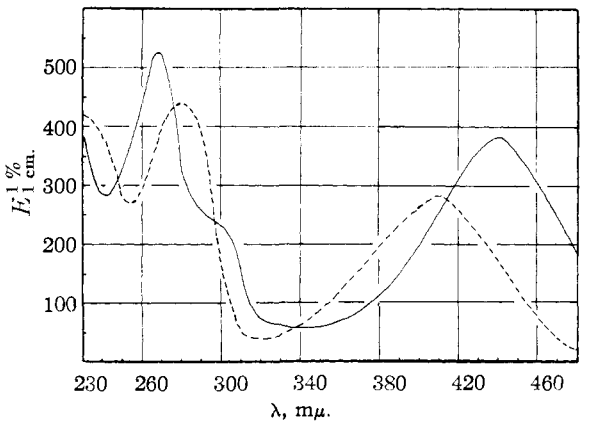

Fig. 1.—Ultraviolet absorption spectrum of the yellow pigment: —, in 0.1 *N* sodium hydroxide; ---, in 0.1 *N* hydrochloric acid.

maxima, at 268 mμ ($E^{1\%}_{1\ cm.}$ 520) and 440 mμ ($E^{1\%}_{1\ cm.}$ 340); in acid solution, there is a shift toward longer wave lengths of the 1st peak (max. at 279 mμ, $E^{1\%}_{1\ cm.}$ 446) and toward the shorter wave lengths of the visible peak (max. at 409 mμ, $E^{1\%}_{1\ cm.}$ 240). The compound has no melting point, but slowly chars on heating. No attempt was made to measure optical activity because of the difficulty of the intense color of even dilute solutions. Its R_f values in various solvents are given in Table I.

TABLE I

Solvent	R_f
n-Propanol–1% ammonia (2:1)	0.44[9]
n-Butanol–acetic acid–water (4:1:5)	.47
Water–isoamyl alcohol	.46
5% Acetic acid	.56
3% Ammonium chloride	.33

As usual with pteridines, considerable difficulty has been experienced in obtaining consistent and reliable analytical figures for carbon, hydrogen and nitrogen, an added complication in this case being the persistence of a non-combustible residue from material obtained from powdered cellulose columns. Early results therefore have given values between the following figures: C, 39–42; H, 4–6; N, 18.6–24.0. On the crystalline material described above the following results have been obtained: C, 41.7; H, 4.3; N, 24.1. This corresponds roughly to $C_{10}H_{12}N_5O_{5.5}$.

The compound is soluble in water (1 mg./ml.) and in aqueous mixtures of various organic solvents, but is insoluble in most organic solvents. The best illustration of this generalization is in its behavior in acetone in which it is completely insoluble, yet in 50% aqueous acetone it is 4 to 5 times more soluble than it is in water.

PASADENA, CALIF.

[CONTRIBUTION FROM THE KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Pteridines from *Drosophila*. II. Structure of the Yellow Pigment[1]

BY H. S. FORREST[2] AND H. K. MITCHELL

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The yellow pigment, occurring principally in the eyes of *Drosophila melanogaster*, which has been isolated in pure crystalline form, has been submitted to a series of degradative experiments and it is concluded from these that the compound has the structure 2-amino-4-hydroxy-7,8-dihydro-8-lactylpteridine-6-carboxylic acid.

In an earlier paper[3] we have described the isolation in crystalline form of a yellow pigment from *Drosophila melanogaster* in which it is concentrated in the eyes and to a lesser extent in the malpighian tubules and testes. A mutant, *sepia*, was used as the source of this compound because it is accumulated in this mutant in much greater amount than in wild-type flies. Its possible significance as an intermediate in the biosynthesis of the red eye pigments of *Drosophila*, and as an important photo-receptor for these flies, has been briefly mentioned. It is the purpose of this paper to present the results of degradative experiments which have led to the tentative assignment of a structure to this compound. Many of these experiments were carried out on material of low purity (as determined by the eventual isolation of crystalline material), but the validity of the conclusions drawn from them has been checked by similar, or, in some cases, simplified experiments on the pure material.

The first clue to the chemical nature of the yellow pigment arose from a study of its photodecomposition products. On exposure to sunlight, it is very rapidly decomposed in dilute alkaline solution, a little more slowly in neutral or acid solution, the color disappearing and an intense blue ultraviolet fluorescence being produced. As revealed by paper chromatography, this fluorescence arises from two components in the solution, but the major one was identified by paper chromatography as 2-amino-4-hydroxypteridine-6-carboxylic acid (I); and this was confirmed by its purification through the disodium salt and comparison of its absorption spectrum in acid and alkaline solution with an authentic specimen of this acid prepared from pteroylglutamic acid[4] and by decarboxylation of, and purification of the products from, both the natural and synthetic acids and com-

(1) These investigations were supported by funds from the Rockefeller Foundation, the Williams–Waterman Fund for the Combat of Dietary Diseases and by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research Contract No. N-6-onr-244, Task Order 5.

(2) U. S. Public Health Service Postdoctoral Fellow during part of the work described.

(3) H. S. Forrest and H. K. Mitchell, THIS JOURNAL, **76**, 5656 (1954).

(4) J. H. Mowat, *et al.*, *ibid.*, **70**, 14 (1948).

parison of their ultraviolet absorption spectra and chromatographic behavior.

The identification of this pteridine as a major part of the molecule of the yellow pigment, combined with its similarity in ultraviolet spectrum (Fig. 1), color, fluorescence and light instability, to riboflavin (III) which as has been pointed out,[5,6] can be considered as a pteridine derivative, indicated a possible close relationship between the two, and a further point of evidence for this resulted from quantitative periodate oxidation of the pigment under the special conditions used for riboflavin.[7]

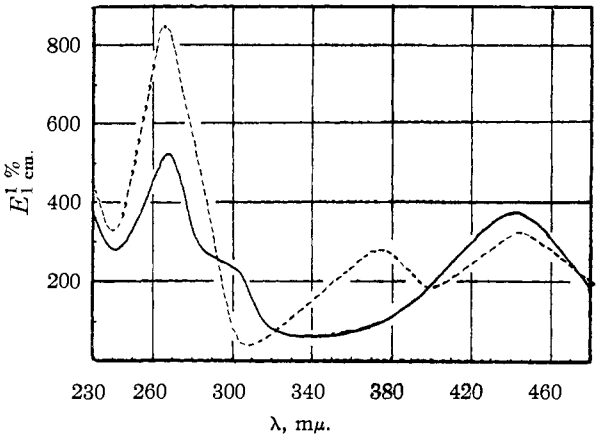
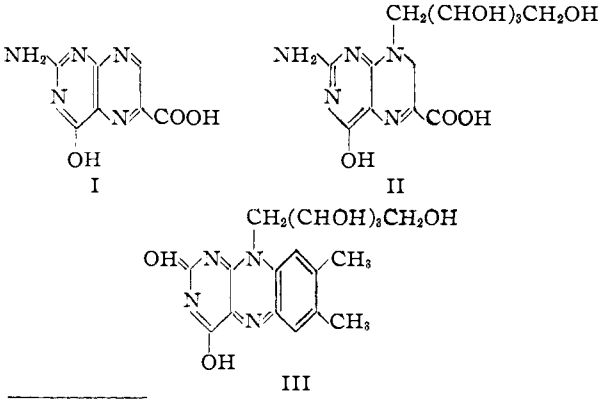


Fig. 1.—Ultraviolet absorption spectra: —, yellow pigment in 0.1 *N* sodium hydroxide; ---, riboflavin at *p*H 7.

Assuming a molecular weight of about 350, a reasonable one on the basis of the figure of 300 given by Maas[8] for the red pigments, results were obtained consistent with an uptake of about 3 moles of periodate during the oxidation, which was very rapid, again corresponding to riboflavin in quantity and speed. The pigment also was found to be oxidized rapidly in alkaline solution by permanganate and the sole fluorescent product was shown to be the acid I which could be readily and accurately measured spectrophotometrically. Such an estimate of the amount of I produced from a known weight of yellow pigment led to the conclusion that a fragment of molecular weight 150 was lost during the oxidation, and moreover this must have been removed from a nitrogen atom (see later). The tentative formula II was thus considered and seemed consistent with the data thus far accumulated.



However, further experiments with periodate, although confirming the uptake of 3 moles/mole, showed that neither formaldehyde nor formic acid was produced by the oxidation whereas II should give two moles of formic acid and one of formaldehyde. Also, the compound was much more labile to acid (complete disappearance of color and fluorescence in 3 hours at 100° with 0.5 *N* hydrochloric acid) as compared to riboflavin which is quite stable under these conditions; thus II had to be abandoned as a representation of the structure of the compound.

It should be mentioned, at this point, that much difficulty was encountered in hydrolytic and degradative experiments because of the complex pattern of products obtained from such experiments. Thus, for example, paper chromatography of an acid hydrolysate revealed the presence of four and sometimes five fluorescent substances, although the main product was the pteridinecarboxylic acid (I). Again, treatment with bromine caused an immediate decolorization of the yellow pigment, about 4 atoms of bromine disappeared, but two blue fluorescent compounds were produced, one of which was the pteridinecarboxylic acid (I) and the other a compound with very similar ultraviolet spectrum but with different R_F values from I (Fig. 2). Hydrogenation in glacial acetic acid or water and with platinum or palladium catalysts resulted in deep seated changes in the molecules and a complex mixture of fluorescent compounds was obtained. As mentioned earlier, two fluorescent compounds were obtained on photo-decomposition, I predominating, however, and alkaline hydrolysis also gave I and two other fluorescent compounds in minor yield.

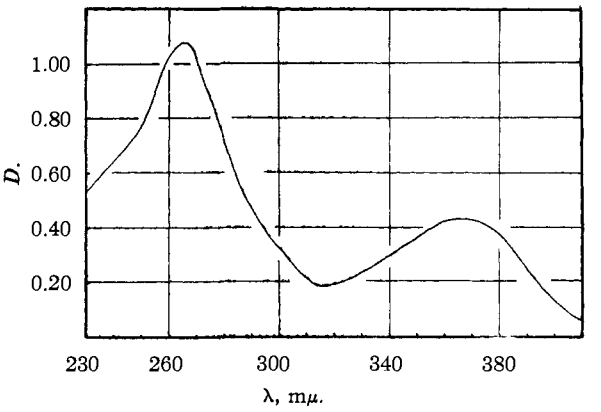


Fig. 2.—Ultraviolet absorption spectrum of the product resulting from the action of bromine on the yellow pigment.

On the other hand, the pigment reacted with hydroxylamine and 2,4-dinitrophenylhydrazine to give an oxime and a dinitrophenylhydrazone and although the analyses of these could only be used as an indication that one carboxyl group was present in the molecule, they were useful in eliminating another possibility. This was that I might be present as the 6-aldehyde in the intact molecule. Hy-

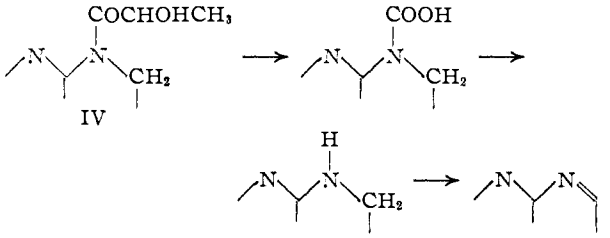
(5) H. S. Forrest, *et al.*, *J. Chem. Soc.*, 3 (1951).
(6) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **75**, 4311 (1953).
(7) H. S. Forrest and A. R. Todd, *J. Chem. Soc.*, 3295 (1950).
(8) W. K. Maas, *Genetics*, **33**, 177 (1948).

drolysis of the oxime with dilute alkali gave I as the chief product, as was also the case with mineral acid hydrolysis of the 2,4-dinitrophenylhydrazone, and thus it was concluded that the carboxyl group existed as such in the molecule. On treatment with acetic anhydride and a trace of perchloric acid, the pigment gave a monoacetyl derivative.

The key to the nature of the side chain came from a re-examination of the periodate oxidation. Since experiments on the purification of the pigment had been carried on simultaneously and had finally resulted in the isolation of a crystalline product, it was now possible to use this pure material. The same value for the periodate uptake was obtained on this, and the same negative results in tests for formaldehyde and formic acid, but a test for acetaldehyde[9] was positive, and a quantitative colorimetric determination of this showed that 1 mole was liberated per mole of pigment. Further, repetition of the quantitative permanganate oxidation experiments on this pure material resulted in a lowering of the estimates of the molecular weight of the oxidized fragment from 150 to about 70.

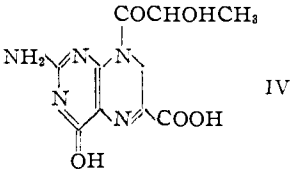
The presence, therefore, of a periodate oxidizable group containing the CH_3CHO system, a ketonic group, a molecular weight of this order and the formation of a monoacetyl derivative, pointed strongly to its formulation as $CH_3CHOHCO-$, *i.e.*, a "lactyl" group. Confirmation of this was obtained by paper chromatographic identification of lactic acid in acid or alkaline hydrolysates or in the products from photo-decomposition and by the appearance of a positive color test for lactic acid[10] after the same decompositions. Because of the small amounts of material available, however, it has not been possible to prepare and characterize a derivative of lactic acid and, to this extent, this formulation is tentative.

On the basis of a partial structure, such as IV in which the lactyl group is presumed to be joined to one of the nitrogen atoms of the pteridine nucleus, thus forming a dihydropteridine derivative, some of the complications of the degradative experiments can be explained. Thus on a molecular weight of 280 (I plus $CH_3CHOHCO-$), the molar periodate consumption drops to about 2.4 and it seems likely, since the only isolable pteridine from such an oxidation is I, that one mole is used for the oxidation of the side-chain, leaving an N-carboxylic acid which as a disubstituted urethan might be expected to decarboxylate spontaneously, and a second mole of periodate would then be used for the oxidation of the resultant dihydropteridine, *i.e.*



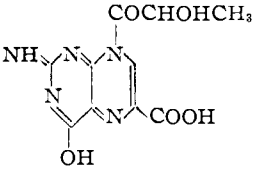
The action of bromine must be explained as, in the first place, one of oxidation, followed possibly by a ring substitution. With a molecular weight of about 280, the consumption of hydrogen corresponds to about 2 moles/mole one of which would be taken up by the keto group and the second by the ring to give a tetrahydropteridine. At this stage, presumably, disruption of the molecule could take place since tetrahydropteridines are known to be unstable unless they contain a substituent on N^5.[11]

There remains then the question of the position of attachment of the lactyl group to the pteridine nucleus. The possibility that this might be at any of the carbon atoms has already been eliminated by the results of permanganate oxidation when only I is obtained. The ultraviolet spectrum would indicate that it is attached to the pyrazine ring and this leaves two possibilities, N^5 or N^8. Biologically there are analogies for substituents in either position: the N^8 as in riboflavin, the N^5 as in "folinic acid SF"[12] (leucovorin[13]). Comparison of the properties of the yellow pigment with those of riboflavin already has been made, and the similarities have been commented on. On the other hand, "folinic acid SF" has a very different absorption spectrum (max. 242 mμ); moreover, the formyl group is remarkably stable to alkali, in marked contrast to the acyl group of the yellow pigment. Also, in general, 7,8-dihydropteridines (although in some cases these have not been rigidly proved to be such) have absorption maxima in the 265–270 mμ region, which shift in acid solution to slightly longer wave lengths; again a substituent (such as a formyl group) on position N^5 has been shown to increase the intensity of the ultraviolet absorption by as much as 50%,[11] yet the molecular extinction of the yellow compound is almost the same as that of I. Thus although direct evidence is not available and will be difficult to obtain because of the instability of the side chain in its attachment to the pteridine ring, the indirect evidence favors its location at N^8 and we therefore propose tentatively the following structure[13a] for the yellow pigment



IV

(9) *Cf.* R. J. Block and D. Bolling, *J. Biol. Chem.*, **130**, 365 (1939).
(10) B. F. Miller and J. A. Muntz, *ibid.*, **126**, 413 (1938).
(11) A. Pohland, *et al.*, THIS JOURNAL, **73**, 3247 (1951).
(12) M. May, *et al.*, *ibid.*, **73**, 3067 (1951).
(13) D. B. Cosulich, *et al.*, *ibid.*, **74**, 3252 (1952).
(13a) A modification of the structure proposed herein was suggested to us by Dr. H. C. S. Wood at the Ciba Conference on the "Chemistry and Biology of Pteridines" held in London in March, 1954 (at which a general account of the work described above was presented). This was that a "dehydro" derivative of IV might better represent the compound and explain its properties, *i. e.*



This provides a good explanation of its general resemblance to riboflavin (which has an analogous system of conjugated double bonds) and is not in conflict with any of the evidence presented above.

Experimental

Paper Chromatography.—Throughout this work, paper chromatography has been used as the principal method of identifying compounds and as a general test of purity. All of the pteridines encountered herein have either been visibly colored or have been very markedly fluorescent in ultraviolet light (Keese lamp, principal emission at a wave length of 360 mμ). Conditions and methods were the same as described previously,[14] except that no very rigid control was kept of temperature or time of running, known compounds always being run at the same time as controls. Other solvent systems beside the routine one of *n*-propanol, 1% ammonia (2:1) are mentioned specifically as they were used.

As recorded,[3] the following figures were obtained on the crystalline compound, dried at 120° *in vacuo*.

Anal. Calcd. for $C_{10}H_{11}O_5N_5$: C, 42.7; H, 4.0; N, 24.9. Calcd. for $C_{10}H_{11}O_5N_5 \cdot {}^1/_2H_2O$: C, 41.4; H, 4.1; N, 24.1. Found: C, 41.7; H, 4.1; N, 24.1.

Isolation of I from Photodecomposition.—The yellow pigment (20 mg.) was dissolved in 1% aqueous ammonia (20 ml.) and the solution was allowed to bleach in sunlight in a shallow layer in a Petri dish. After the disappearance of the yellow color, the solution was evaporated *in vacuo* to small bulk and then acidified with dilute acetic acid. The greyish precipitate was collected and recrystallized twice from a mixture of 5% acetic acid and an equal volume of ethanol and then as its sodium salt from 2 *N* sodium hydroxide. This was reconverted to the acid by dissolution in boiling water and acidification. The precipitated material (5 mg.) was collected and compared with authentic I in its ultraviolet absorption spectrum at *p*H 1 and *p*H 13, and in its chromatographic behavior (Table I).

Decarboxylation.—The above material (5 mg.) was heated at 250–300° for 3 hours at the same time as an authentic specimen of I. The resulting crude products, dissolved in dilute ammonia, were applied to strips of filter paper (30 × 17 cm.) and the decarboxylation products were separated from unchanged starting materials by irrigating these with the propanol–ammonia solvent.

Elution of the required bands from the paper with water and comparison of the ultraviolet absorption spectra at *p*H 1 and at *p*H 13, and R_F values (Table I) of the two samples confirmed their identity.

TABLE I

Solvent	Acid (I)	Photolysis prod.	Decarboxylated cmpd. From acid, I	Decarboxylated cmpd. From photolysis
sec-Butyl alc., formic acid, water (8:2:5)[15]	0.42	0.42	..	..
n-Propanol, *N* hydrochloric acid (2:1)	.30	.30	..	..
n-Butanol, acetic acid, water (4:1:5)	.22	.22	0.27	0.27
n-Propanol, pyridine, water (5:3:2)	.07	.07	..	..
n-Propanol, 1% ammonia (2:1)	.14	.14	.44	.44
5% Acetic acid	.47	.47	.65	.65

Periodate Oxidation.—The yellow pigment (20 mg.) was dissolved in sodium metaperiodate solution (10 ml., 0.026 *M*) at 0° and aliquots were removed and titrated at various time intervals. The result is shown in Fig. 3.

With a molecular weight of 280, the consumption of periodate = 2.4 moles/mole.

Formaldehyde was determined by the modified[16] Boyd and Logan[17] procedure which is quantitatively sensitive down to 2 μg. of formaldehyde. None could be detected.

Formic acid was estimated by reduction with magnesium and hydrochloric acid to formaldehyde[18] and determination

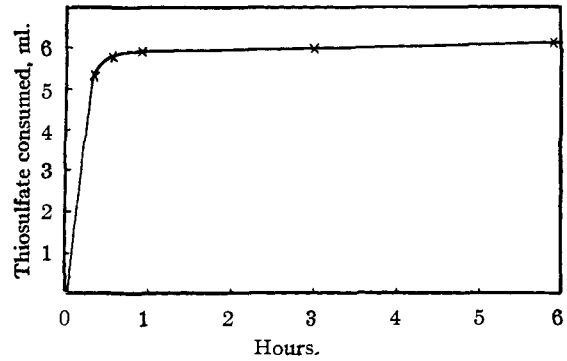


Fig. 3.—Periodate oxidation of the yellow pigment, each aliquot containing 3.6 mg.

of this with chromotropic acid (attempts to develop a micro method based on the liberation by formic acid of iodine from iodate–iodide or on its titration with barium hydroxide after micro diffusion in a Conway unit were not successful). This method is reasonably satisfactory for known amounts (2–20 μg.) of formic acid although it is much less sensitive than the formaldehyde method because of the poor yield in the reduction. Unfortunately also, if formaldehyde is present, the results are much more variable due to the large losses of formaldehyde suffered in the reduction. Nevertheless good results (2 moles/mole of formic acid; 0.8 mole/mole formaldehyde) were obtained on a test run with riboflavin using a total quantity of 0.21 mg. of material. The complications due to formaldehyde were not present in the estimation of formic acid from the yellow pigment and from an aliquot (containing 70 μg.) of a periodate oxidation mixture, treated with magnesium and hydrochloric acid under standard conditions, a yield of 2 μg. of formic acid was obtained. On a molecular weight of 280, the theory for 1 mole of formic acid per mole is 10.5 μg.

Acetaldehyde produced by periodate oxidation was measured by a combination of the methods used by Block and Bolling[9] and Shinn and Nicolet[19] for the estimation of threonine. Thus aliquots of a threonine solution (1 mg./ml.) were decomposed with sodium metaperiodate (0.5 *M*), the acetaldehyde was aerated into a solution of *p*-hydroxydiphenyl (10 mg.) in sulfuric acid (10 ml.) and the color developed was estimated at 560 mμ in a Beckman spectrophotometer. The standard straight line plot thus obtained was used in the assay of a solution of the yellow pigment (0.426 mg.) treated with periodate. A value of 67 μg. of acetaldehyde was obtained. Using a mol. wt. of 280, the theory for 1 mole of acetaldehyde per mole of compound is 67 μg.

The fluorescent product from periodate oxidation was purified and identified in the following way. After titration with sodium thiosulfate of a periodate-oxidized solution (2 ml.) of the substance (4 mg.), the whole (*ca.* 15 ml.) was passed through a small column (4 × 0.5 cm.) of acid-washed "Superfiltrol," the blue fluorescent substance being adsorbed at the top of the column. The column was washed thoroughly with water and the fluorescent compound was washed out with 25% acetone. The eluate was then evaporated and the residue was identified as I by its paper chromatographic behavior in 3 solvents, propanol–ammonia, 3% ammonium chloride,[20] R_F, 0.54 and propanol, 5% acetic acid (2:1), R_F, 0.27.

Permanganate Oxidation.—An aqueous solution (0.1 ml.) of the crystalline yellow pigment (85 μg.) was treated with sodium hydroxide solution (0.1 ml., 0.1 *N*) and immediately with saturated potassium permanganate (0.02 ml.) and the whole was then heated at 100° for 15 minutes. Excess permanganate was destroyed with formaldehyde and the precipitated manganese dioxide was washed thoroughly (3 times) by centrifugation with sodium hydroxide solution (0.1 *N*). The supernatants were combined and made up to 5 ml. In a parallel experiment the manganese dioxide was washed with 1% ammonia to avoid accumulation of inorganic material, and the combined supernatants were then

(14) E. Hadorn and H. K. Mitchell, *Proc. Natl. Acad. Sci.*, **37**, 650 (1951).

(15) F. Weygand, *et al.*, *Experientia*, **VI**, 184 (1950).

(16) B. N. Ames, H. K. Mitchell and M. B. Mitchell, THIS JOURNAL, **75**, 1017 (1953).

(17) M. J. Boyd and M. A. Logan, *J. Biol. Chem.*, **146**, 279 (1942).

(18) W. M. Grant, *Anal. Chem.*, **20**, 267 (1948).

(19) L. A. Shinn and B. H. Nicolet, *J. Biol. Chem.*, **138**, 91 (1941).

(20) A. G. Renfrew and P. C. Platt, *J. Amer. Pharm. Assoc.*, **39**, 657 (1950).

streaked on a sheet of filter paper (22 × 17 cm.) and this paper developed with the propanol–ammonia solvent. The only blue fluorescent band (R_F, 0.15) was eluted with water and the eluate was made up to 5 ml. with sodium hydroxide solution (0.1 *N*).

The absorption spectra of these two samples corresponded exactly to that of I, and its concentration was determined using the known constants for either the 262 mμ or the 365 mμ peak. In the first determination the amount of acid was 65 μg. and in the second, 64 μg. The molecular weight of I is 208, and hence the molecular weight of the original pigment must be about 280.

Acid Hydrolysis.—A solution (2 ml.) of the yellow pigment (1.74 mg.) was treated with hydrochloric acid (2 *N*, 0.5 ml.) and the whole heated on the water-bath for 3 hours. The solution was then evaporated *in vacuo* to dryness and the residue redissolved in 1% ammonia (2 ml.). An aliquot (0.1 ml.) made up to 5 ml. was used to determine the amount of pteridine present by measurement of its ultraviolet absorption at 360 mμ. This amount was found to be 1.03 mg. The absorption peak in the near ultraviolet was found to be a broad one extending over the wave lengths 266 to 274 mμ, instead of being at 262 mμ as it should be for I. Paper chromatography revealed the presence of 4 fluorescent materials, I and 3 others, R_F's 0.32, 0.41 and 0.47 (in propanol–ammonia) in smaller amount. From the ultraviolet absorption spectrum these would appear to be dihydropteridines. Hydrolyses for shorter times or with more dilute acid produced even more complex mixtures. Refluxing the pigment with Dowex 50 in the acid form for 30 minutes also was effective in destroying the pigment, and the blue fluorescence produced was almost completely adsorbed on the resin, but difficulty was experienced in quantitative elution of it from this, although again I was washed off using hydrochloric acid (4*N*) and identified in the usual way.

Action of Bromine. (a) Estimation of Bromine Uptake.—A standard solution (0.2 ml., 0.01 *N*) of bromate–bromide was treated *in vacuo* with sulfuric acid (10%, 0.1 ml.) and after 10 minutes excess potassium iodide solution was added and the liberated iodine was titrated with sodium thiosulfate. A sample of yellow pigment (1.005 mg.) and the same quantity of bromate–bromide were then similarly treated and the difference in titration gave the amount of bromine consumed (1.2 mg.). With molecular weight of 280 this is equivalent to about 4 atoms. (b) Estimation of **PTERIDINES PRODUCED.**—A solution (0.1 ml.) of yellow pigment (0.0852 mg.) was streaked on a sheet of filter paper (30 × 17 cm.) and this streak was overlaid with a saturated aqueous solution of bromine, until the yellow color was completely destroyed. After drying, the paper was run chromatographically using propanol–ammonia as the solvent and the two bands which separated were eluted with water and the eluates made up to 5 ml. with sodium hydroxide solution (0.1 *N*). The slower running component (R_F, 0.14) was identified as I and the quantity estimated in the usual way by its ultraviolet absorption spectrum. The yield was 0.015 mg. The faster moving band, R_F 0.41, had maxima at 265 and 360 mμ (Fig. 2) and assuming it to be closely related to I, there was calculated to be about 0.055 mg. of it produced.

Hydrogenation.—With Adams catalyst in glacial acetic acid (5 cc.) the pigment (0.946 mg.) absorbed 1.97 ml. of hydrogen, corresponding to 4 moles of hydrogen (based in a molecular weight of 280) but paper chromatography revealed that a complex mixture of products resulted. With palladous oxide, the quantity of hydrogen taken up by an aqueous solution (5 ml.) of pigment (7.13 mg.) was 0.99 ml. (theory for 2 moles of hydrogen, 1.14 ml.). Paper chromatography (propanol–ammonia) of the solution after removal of the catalyst, revealed the presence of four fluorescent compounds, a little of the original starting material, a blue one R_F 0.32, a yellow one R_F 0.39, and another blue one R_F 0.46.

Formation of the Oxime.—A solution (5 ml.) of yellow pigment (5 mg.) was treated with sodium acetate (30 mg.) and hydroxylamine–hydrochloride (20 mg.) on the water-bath for 30 minutes. It was then evaporated to cloudiness *in vacuo*, cooled and the yellow precipitate (4 mg.) was collected and recrystallized from a small volume of dilute acetic acid, from which it separated as a microcrystalline yellow solid. It had an R_F in butanol, acetic acid, water (4:1:5) of 0.57 and in propanol–ammonia of 0.56, and exhibited a light blue fluorescence in alkaline solution, yellow in acid. Alkaline hydrolysis (0.1 *N* NaOH for 2 hours at 100°) caused its partial decomposition to I. Its absorption spectrum showed peaks at 265 mμ ($E^{1\%}_{1\text{ cm.}}$ 500) and 380 mμ ($E^{1\%}_{1\text{ cm.}}$ 345) in 0.1 *N* sodium hydroxide solution, and at 268 mμ ($E^{1\%}_{1\text{ cm.}}$ 420) in 0.1 *N* hydrochloric acid solution.

Anal. Calcd. for $C_{10}H_{12}N_6O_5$: C, 40.5; H, 4.05. Found: C, 40.7; H, 4.8.

Formation of 2,4-Dinitrophenylhydrazone.—A solution of the pigment (15 mg.) in dilute acetic acid was treated with a filtered excess of 2,4-dinitrophenylhydrazine in perchloric acid. After 30 minutes the brick red precipitate which slowly formed was collected and dried. It was insoluble in water or organic solvents, although it dissolved in pyridine to give a deep red solution.

Anal. Calcd. for $C_{16}H_{15}N_9O_8$: C, 41.2; H, 3.3. Found: C, 41.2; H, 3.9.

When heated at 100° with hydrochloric acid (2.5 ml., 0.5 *N*) for 6 hours this compound (3 mg.) gave an insoluble residue (1 mg.) which was removed and the resulting solution after reduction in volume was examined by paper chromatography. The pattern of fluorescent spots was identical with that resulting from the hydrolysis of the original compound, I predominating.

Acetylation.—The yellow pigment (15 mg.) suspended in acetic anhydride (0.5 ml.) was treated with a drop of 70% perchloric acid, resulting in a clear solution. This was allowed to stand for 30 minutes and then treated with water (2 ml.) and cooled. The precipitate was collected by centrifugation, dissolved in water and the solution was passed through a small (5 × 1 cm.) column of "Filtrol Grade 58." The yellow, adsorbed material was washed out with 25% aqueous acetone and this fraction was collected, evaporated to small volume and crystallized from 5% acetic acid, to give a micro-crystalline yellow solid (5 mg.), R_F in butanol, acetic acid, water (11:3:4), 0.71.

Anal. Calcd. for $C_{12}H_{13}N_5O_6$: C, 44.6; H, 4.03; N, 21.7. Found: C, 44.3; H, 4.3; N, 21.6.

Characterization of Lactic Acid. (a) Acid Hydrolysis.—The yellow pigment (5 mg.) was heated in glacial acetic acid (0.15 ml.) for 3 hours at 100°, during which time the yellow fluorescence was completely destroyed. The reaction mixture was centrifuged to remove a buff-colored precipitate (3.5 mg.) which was shown to be largely I by chromatography, and the supernatant liquid and washings from this precipitate were combined and evaporated in a desiccator over sodium hydroxide. The residue was dissolved in water and run on a paper chromatogram in the solvent system used by Kennedy and Barker[21] for separating aliphatic carboxylic acids, along with a control specimen of lactic acid. On spraying the dried paper with indicator, blue spots were obtained with identical R_F values from both samples. However, the spot from the acid hydrolyzed pteridine was not very strong and it was concluded, that there must be a considerable loss of even the relativley nonvolatile lactic acid during the evaporation of the acid hydrolysate.

(b) Ammonia Hydrolysis or Light Decomposition in 1% Ammonia.—The yellow pigment (5 mg.) was treated with concd. ammonia (0.3 ml.) and the solution was carefully heated to 100°, then stoppered and kept at this temperature for 1 hour. At the end of this time it was evaporated to dryness and the residue was washed with a small quantity of water; this aqueous solution was used for chromatography. At the same time another sample of the yellow pigment (5 mg.) was decomposed by light in 1% ammonia solution (0.5 ml.) and after evaporation, the residue was treated in the same way. Paper chromatography and spraying with indicator again revealed the presence of material from both these decompositions with an R_F value identical with that of lactic acid, in 95% ethanol, concd. ammonia (100:1),[22] R_F 0.58.

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