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

Pteridines from Drosophila. III. Isolation and Identification of Three More Pteridines[1]

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RECEIVED APRIL 4, 1955

A new compound has been isolated from *wild type* Drosophila and characterized by degradative reactions as 2-amino-4-hydroxy-6-(1',2'-dihydroxypropyl)-pteridine. This structure has been confirmed by synthesis. 2-Amino-4-hydroxypteridine and isoxanthopterin have also been isolated and identified by comparison with synthetic specimens.

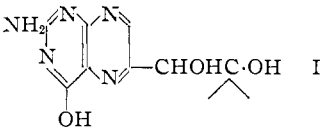
In previous papers,[2] we have given details of the isolation of a yellow pigment from Drosophila and have tentatively identified it as N^8-lactyl-7,8-dihydro-2-amino-4-hydroxypteridine-6-carboxylic acid. Running along with this compound, in the chromatographic solvent (*n*-propanol, 1% aqueous ammonia) used by Hadorn and Mitchell,[3] is a blue fluorescent spot (named by them, Fl 4) which separates readily from the yellow compounds, and can be divided into two compounds in other solvent systems. It is the purpose of this paper to describe the isolation and chemical identification of these two components, and of another purple fluorescent material (originally called Fl 3) found chiefly in the testes of the adult flies.

In the case of the yellow pigment, the task of identification was made easier by the fact that relatively large quantities of the material could be isolated from a mutant, *sepia*, which accumulates it in large excess. No such advantageous circumstances obtain with the blue fluorescent materials or the purple fluorescent substance to be described in this paper, and chemical identification has thus been carried out on very small amounts of material, usually contained in dilute aqueous solution. Convincing evidence on their structure has, however, been obtained and confirmed by comparisons with synthetic materials.

"Fl 4" was first obtained as a by-product in the purification of the yellow compound, previously described, and of the red pigments (experiments on the purification of which will be described later). In brief, it was isolated from the combined eluates of a number of chromatographic columns, by adsorption on charcoal and elution from this with aqueous pyridine. At this point, it was highly contaminated with 2-amino-4-hydroxypteridine-6-carboxylic acid, arising chiefly from unavoidable light decomposition of the yellow pigment, but after chromatographic separation from this, it was obtained as a pale buff colored, microcrystalline solid.

It soon became apparent, however, that this material was not homogeneous and, indeed, using a different irrigating solvent (dil. acetic acid) it was readily separated into two components. In addition to their differing R_F values in this solvent, these were distinguished by their behavior toward alkaline permanganate. The first (hereinafter referred to as Fl 4A) was readily oxidized, whereas the second (Fl 4B) was quite unaffected by this reagent. Their absorption spectra on the other hand, were almost identical. The elucidation of the constitution of these compounds will be dealt with in turn.

Fl 4A, then, in pure form was oxidized completely by permanganate to give a single fluorescent substance. This was identified as 2-amino-4-hydroxypteridine-6-carboxylic acid from its absorption spectrum and by chromatographic comparison with a synthetic specimen. By estimation of the amount of this acid produced from a known amount of Fl 4A, it was deduced that its molecular weight was about 220–230. Fl 4A was also oxidized by sodium metaperiodate and again the only identifiable reaction product was the 6-carboxylic acid. However, a more detailed study of the reaction was now made. The molecular consumption of periodate was shown to be 1 mole/mole (using a molecular weight of 230 as derived from the permanganate oxidation experiment) and the immediate absorption spectrum of the product, although somewhat difficult to measure due to the high absorption of sodium metaperiodate and periodate in the near ultraviolet, showed bands corresponding to 2-ammino-4-hydroxy-6-aldehydopteridine. This latter has a very characteristic absorption spectrum, quite different from other 6-substituted pteridines.[4] Furthermore a solution of this degradation product, obtained by absorbing it from the periodate oxidation mixture on charcoal and eluting from this with 30% aqueous pyridine, gave a precipitate with 2,4-dinitrophenylhydrazine. These observations gave a partial structure I, for Fl 4A.



I

There remained to be determined the nature of the substituents on the second carbon atom of the side chain. In our early work, a test for formaldehyde on the periodate oxidation products was positive, although the yield was only about 0.5 mole/mole. This led to the supposition that the side chain was a dihydroxyethyl one, but synthesis of this compound by condensation of erythrose (D or L) with 2,4,5-triamino-6-hydroxypyrimidine in the presence of hydrazine, and comparison of the synthetic with the natural material, disproved this theory.

(1) These investigations were supported by funds from the Rockefeller Foundation, the Williams–Waterman Fund for the Combat of Dietary Diseases, a research grant from Merck and Co., Inc., 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.

After this communication was submitted for publication the Editors informed us that Patterson, Broquist, Albrecht, von Saltze and Stokstad (THIS JOURNAL, **77**, 3167 (1955)) have isolated from urine, a pteridine which appears to be the same as one of these described here.

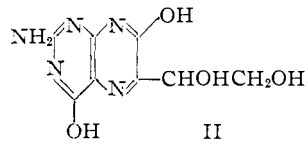
(2) H. S. Forrest and H. K. Mitchell, THIS JOURNAL, **76**, 5656, 5658 (1954).

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

(4) C. W. Waller, *et al.*, THIS JOURNAL, **72**, 4630 (1950).

However, another possibility in keeping with the evidence so far presented, would be a side chain containing an amino group in place of one of the hydroxyl groups. Mild treatment with nitrous acid, sufficient to convert such an amino group to the hydroxy compound without affecting the 2-amino group of the pteridine nucleus, left the Fl 4A unaltered except for a small amount of oxidation to the pteridine-6-carboxylic acid. Furthermore the formaldehyde production could not be confirmed on very carefully purified material.

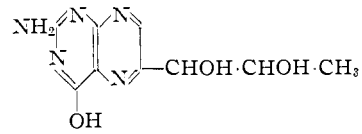
A very close analogy to the state of affairs reached in the elucidation of the structure of Fl 4A is provided by the work of Tschesche and Korte[5] on the structure of fluorescyanine obtained from the eggs and chrysalis of the silk worm. They postulated a glycol side chain (*cf.* II) based on its rupture with periodate to the corresponding aldehyde



II

and succeeded in synthesizing the compound II only to find that it was not identical with the natural product.

A further possibility was now tested, namely, that acetaldehyde was being produced by periodate oxidation. The micro-method described in our previous paper[2] was employed, and a yield of about 0.8 mole/mole of acetaldehyde was obtained. Fl 4A, therefore, on periodate oxidation, gave 2-amino-4-hydroxy-6-aldehydopteridine and acetaldehyde with the consumption of 1 mole of periodate and its structure could be written with certainty as



One further reaction of the compound should be mentioned. In neutral solution, it decomposes in sunlight in characteristic fashion to give probably as the first product 2-amino-4-hydroxypteridine-6-carboxylic acid, although experimentally a mixture of this substance and 2-amino-4-hydroxypteridine, which is known to arise from the acid in light, was obtained.

Confirmation for the formula III as the structure of Fl 4A was obtained in the following way. Rhamnotetrose has been described by Fischer[6] who obtained it by a Wohl degradation of tetracetylrhamnonitrile. Using this procedure, a solution of rhamnotetrose was obtained and condensed with 2,4,5-triamino-6-hydroxypyrimidine in the presence of hydrazine. The crude product so obtained was purified on "Filtrol" columns. It crystallized from water or dilute acetic acid in tiny colorless plates. The absorption spectrum was identical with that of the natural product and chromatographically they were indistinguishable (Table II). The synthetic material reacted with periodate giving similar results to those obtained with the natural compound, but with permanganate, a mixture of the 6- and 7-carboxylic acids was obtained. The natural material gave only 2-amino-4-hydroxypteridine-6-carboxylic acid. A product containing a much greater preponderance of the 6-isomers could be obtained by long incubation of the rhamnotetrose with hydrazine, although the yield in the reaction dropped correspondingly.

As outlined above, Fl 4B was isolated from crude Fl 4 as the second component by chromatographic separation using dilute acetic acid as the irrigating solvent. It was obtained as a buff colored microcrystalline solid, but most of the reactions described below were carried out on a dilute aqueous solution. The substance was not oxidized by permanganate or periodate and was quite stable to acid or alkali. It was not affected under acetylating conditions and was unchanged after heating at 300° for 6 hr. Its absorption spectrum showed peaks at 252 and 360 mμ in 0.1 *N* sodium hydroxide, and at 245 and 312 mμ in 0.1 *N* hydrochloric acid.

These properties were consistent with its formulation as 2-amino-4-hydroxypteridine. This compound was thus synthesized by the two known routes from glyoxal and 2,4,5-triamino-6-hydroxypyrimidine sulfate,[7] and by decarboxylation of 2-amino-4-hydroxypteridine-6-carboxylic acid.[8] The products from these two syntheses were identical and indistinguishable from the natural material, Fl 4B. Confirmation of this was obtained by deamination, when the natural and the synthetic materials gave the same product, namely, 2,4-dihydroxypteridine.

A chromatographically pure sample of the purple fluorescent compound, Fl 3, was obtained in the following way. Among the methods studied for the purification of the red pigments from Drosophila has been to adsorb them from a trichloroacetic extract of *wild type* flies on the ion-exchange resin, Dowex 50. If this resin was then washed with water, the resulting solution had a striking purple fluorescence; evaporation, and paper chromatography of the concentrate using dilute acetic acid as solvent, then gave a solution of the pure compound. Measurement of the ultraviolet absorption spectrum of this solution revealed peaks at 254 and 339 mμ in 0.1 *N* sodium hydroxide and at 286 and 340 mμ in 0.1 *N* hydrochloric acid. This spectrum closely resembled the absorption spectrum of isoxanthopterin and a synthetic sample of this material[9] was found to be identical in all respects with the natural product. Shortly after the completion of this identification a report appeared[10] on the identity of the purple fluorescent material of Drosophila with isoxanthopterin.

Finally, it should be pointed out that the ultraviolet absorbing substance which occurs in observable quantity in chromatograms prepared from two larvae,[3] has been shown to be identical with uric acid, although this was thought not to be so, originally.

(5) *Cf.* F. Korte in "The Chemistry and Biology of Pteridines," J. and A. Churchill Ltd., London, 1954, p. 159.

(6) E. Fischer, *Ber.*, **29**, 1377 (1896).

(7) C. K. Cain, M. F. Mallette and E. C. Taylor, THIS JOURNAL, **68**, 1996 (1946).

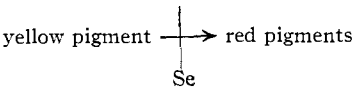
(8) J. H. Mowat, *et al.*, *ibid.*, **70**, 14 (1948).

(9) A. Albert and H. C. S. Wood, *J. Appl. Chem.*, **3**, 521 (1953).

(10) S. Nawa and T. Taira, *Proc. Jap. Acad.*, **30**, 532 (1954).

Discussion

Five different pteridines have now been isolated from Drosophila. These are isoxanthopterin, 2-amino-4-hydroxypteridine, 2-amino-4-hydroxypteridine-6-carboxylic acid, 2-amino-4-hydroxy-6-(1′,2′-dihydroxypropyl)-pteridine and N^8-lactyl-7,8-dihydro-2-amino-4-hydroxypteridine-6-carboxylic acid. Although there are obvious structural relations between these compounds it is impossible, at this stage, to arrange them in an order which might have biological significance. We have shown that the red pigments contain 2-amino-4-hydroxypteridine-6-carboxylic acid in their molecular structure and hence the scheme



can be written with some certainty, but the preceding steps in the biosynthetic pathway cannot be specified at the moment. It is interesting to consider that as the larvae of Drosophila change to pupae, the amount of uric acid present seems to drop, at least on a qualitative level of measurement, *i.e.*, from 2 fully developed larvae, uric acid absorption is plainly visible in ultraviolet light (max. wave length, 260 mμ), whereas this is not so when adult flies are similarly crushed and chromatographed.

It is thus possible to visualize the conversion of uric acid, say, to isoxanthopterin,[11] and the further conversion of this through a fluorescyanine-type structure to Fl 4A. This could then be degraded to the pteridine-6-carboxylic acid, which would be a logical precursor of the yellow pigment and hence the red ones; at the same time, the 6-carboxylic acid might be decarboxylated to 2-amino-4-hydroxypteridine which could have a function of its own or might be incorporated into some of the pigments of yet undetermined structure.

Another possible mode of origin of these pteridines, which must be kept in mind, could be through a series of degradative reactions of riboflavin which although apparently necessary for the nutrition of Drosophila, occurs in very small quantity, if at all, in the adult fly.

The often stated view[12] that the synthesis of the brown pigments is intimately connected with that of the red ones could be reconciled with their chemical dissimilarity, if one of the pteridines acted as a coenzyme or moderator of enzyme reactions connected with the synthesis of the brown pigments. Isoxanthopterin has been implicated in melanogenetic reactions[13] and would fit this role admirably.

These remarks are speculative and much work remains to be done to substantiate or amend them, and to discover the correct interrelations among the pteridines described in this paper.

Addendum.—Since this paper was submitted, we have received notice of a preliminary publication by M. Viscontini, M. Schoeller, E. Loeser, P. Karrer and E. Hadorn, *Helv. Chim. Acta*, **38**, 397 (1955), describing the isolation of riboflavin, and two pteridines (HB_1 and HB_2) from *D. melanogaster*. From a personal communication received from Professor E. Hadorn there is little doubt that HB_1 is the same as our Fl 4B, namely, 2-amino-4-hydroxypteridine, and that HB_2 is our Fl 4A, 2-amino-4-hydroxy-6-(1′,2′-dihydroxypropyl)-pteridine.

(11) For a short account of the conversion of purines to pteridines see A. Albert, *Biochem. J.*, **57**, x (1954).

(12) *Cf.* B. Ephrussi, *Cold Spring Harbor Symposia Quant. Biol.*, **10**, 40 (1942).

(13) M. Polonovski, R. G. Busnel and A. Baril, *Compt. rend.*, **231**, 1572 (1950).

Experimental

Isolation of the Mixture Fl 4.—In a previous paper,[2] we have described the occurrence of a blue fluorescent band as the first fraction from powdered cellulose columns, primarily concerned with the purification of the yellow pteridine. The eluates of this band from a number of columns were treated with charcoal to adsorb all the fluorescent material; the charcoal was then thoroughly washed with water and the blue fluorescence was eluted from it with 30% aqueous pyridine. The pyridine extract was evaporated *in vacuo* and the resulting solid was collected and stored. In a similar manner, this fraction was collected as a by-product from powdered cellulose columns used for purifying the red pigments.

One hundred and eighty mg. of this crude mixture was dissolved in 1% aqueous ammonia and the solution streaked on 4 sheets (50 × 40 cm.) of Munktell No. 50 filter paper. These were then irrigated with propanol, 1% aqueous ammonia (2:1) and after allowing the chromatogram to dry the appropriate band corresponding to the substance in the fly was eluted. A second purification was carried out in the same way. The eluate was then concentrated to dryness *in vacuo* and the residue was crystallized from dilute acetic acid (5%), to give a pale buff colored solid (16 mg.). The ultraviolet absorption spectrum showed maxima at 255 and 365 mμ in 0.1 *N* sodium hydroxide (ratio of optical density at 255 mμ to density at 365 mμ, 3.2).

Further Purification of Fl 4.—The material (16 mg.) described above was redissolved in dilute (1%) ammonia and again streaked on Munktell filter paper (2 sheets; 50 × 40 cm.). The solvent, this time, was 5% dilute acetic acid which gave a fairly good resolution of the two components. Each band was eluted as before, and rechromatographed. The final eluate, in each case, was evaporated to dryness and the residue was crystallized from dil. acetic acid, giving about 3 mg. of Fl 4A, as a buff colored precipitate and about the same quantity of Fl 4B.

The absorption spectrum of Fl 4A is given in Fig. 1.

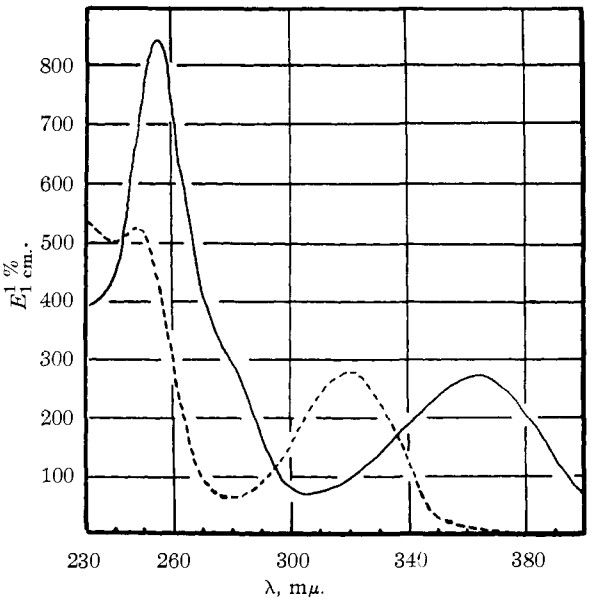


Fig. 1.—Ultraviolet absorption spectrum of Fl 4A: —, in 0.1 *N* sodium hydroxide; - - - -, in 0.1 *N* hydrochloric acid.

Its chromatographic behavior is indicated in the various tables throughout this paper. It has a light blue fluorescence in neutral or alkaline solution, diminished somewhat

in dilute acid solution. At no time was sufficient material available for analysis.

Permanganate Oxidation.—A solution (0.04 ml.) of Fl 4A was treated with sodium hydroxide solution (0.1 *N*; 3 ml.) (estimation of the optical density of this at 255 mμ gave its Fl 4A content, *i.e.*, 7.73 μg./ml.) and then with saturated potassium permanganate (0.04 ml.). After heating at 90° for 30 min., excess permanganate was destroyed by addition of a little alcohol, and the cooled mixture was filtered through Hyflo-Supercel. The absorption spectrum of the filtrate corresponded exactly to that of 2-amino-4-hydroxypteridine-6-carboxylic acid and the identity of the oxidation product was confirmed by paper chromatographic comparison with an authentic sample. Using the known constants for this acid, the solution was shown to contain 7.2 μg./ml. of 2-amino-4-hydroxypteridine-6-carboxylic acid and this arose from 7.73 μg./ml. Hence, the molecular weight of Fl 4A was about 225.

Periodate Oxidation.—A solution of Fl 4A (0.4 mg.) in water (2 ml.) was treated with sodium metaperiodate solution (0.02 *M*, 1 ml.). After 15 min., the undestroyed periodate was estimated by titration of the iodine liberated in boric acid buffer by potassium iodide. With a molecular weight of 230, the consumption of periodate = 0.9 mole/mole.

Formaldehyde was determined using the procedure previously described.[2] A solution containing 40 μg. of the substance gave no color with chromotropic acid.

The product from a periodate oxidation of 0.4 mg. of substance was adsorbed on charcoal (0.5 g.) and, after thorough washing of this, was eluted from itus ing 30% aqueous pyridine. This eluate was evaporated, and the residue was taken up in water. A small amount was used to determine the absorption spectrum which had peaks at 255, 275 and 365 mμ in 0.1 *N* sodium hydroxide. The remainder was treated with 2,4-dinitrophenylhydrazine in perchloric acid, when it gave an immediate orange precipitate.

If the oxidation was carried on for some time, *e.g.*, overnight, the resulting product as identified by chromatography as 2-amino-4-hydroxypteridine-6-carboxylic acid.

Acetaldehyde was determined by the procedure used in the previous paper,[2] using the color developed with *p*-hydroxydiphenyl in sulfuric acid. From a standard curve obtained by oxidation of known amounts of threonine, a sample of the material (80 μg.) was estimated to give 10 μg. acetaldehyde. With a molecular weight of 230, this represents 0.75 mole/mole.

Comparison of Fl 4A with Synthetic 2-Amino-4-hydroxy-6 and/or 7-Dihydroxyethylpteridines.—Sirupy erythrose (both D and L) was condensed with 2,4,5-triamino-6-hydroxypteridine in the presence of hydrazine.[14] The crude condensation products were recrystallized once from water and then compared with Fl 4A and with various other pteridines containing hydroxyalkyl side chains synthesized in the same way. The results are shown in Table I.

TABLE I

Compound	*n*-Propanol, 1% NH_3 (2:1)	Butanol, acetic acid, water (4:1:5)	3% NH_4Cl	5% Boric acid
R = CH_2OH	0.38	0.31	0.54	0.60
R = $CHOHCH_2OH$	.34	.32	.64	.72
	.46	.42		
R = $(CHOH)_2CH_2OH$	.31	.28	.67	.72
	.41	.34		
R = $(CHOH)_3CH_2OH$	.26	.18	.67	.73
	.33	.27		
Fl 4A	.46	.37	.75	.75

The double spots for the dihydroxyethyl-, trihydroxypropyl- and tetrahydroxybutyl- compounds may arise from the 6- and 7-isomers both of which may be produced in the synthesis. Because of the non-identity of the products with the natural material no attempt was made to separate and identify these compounds.

(14) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 79 (1949).

2-Amino-4-hydroxy-6-(1′,2′-dihydroxypropyl)-pteridine.—Rhamnotetrose diacetamide (2 g.)[6] was hydrolyzed on the water-bath with hydrochloric acid (1.6 *N*; 12 ml.). The *p*H of the solution was adjusted to 7, its volume reduced to about 8 ml. and hydrazine hydrate (0.43 ml.) was added. After 30 min., 2,4,5-triamino-6-hydroxypyrimidine dihydrochloride (0.71 g.), sodium bicarbonate (0.57 g.) and glacial acetic acid (0.8 ml.) were added in turn, and the whole heated on the water-bath for 45 min. The reaction mixture was then cooled and the precipitate collected, washed with water, alcohol and ether and dried. The yield was about 0.7 g. Chromatographically this material contained 2 blue fluorescent spots, one identical with natural Fl 4A, and the second running slightly ahead of it in alcoholic solvents. This latter was not removed by recrystallization (0.1 *N* hydrochloric acid) or by treatment of an ammoniacal solution of the crude product with charcoal, and reprecipitation with dilute acetic acid. However, a solution of the crude reaction product (200 mg.) in hydrochloric acid (30 ml., 0.1 *N*) was passed through a small column of "Filtrol Grade 58" (2 × 4 cm.). The fluorescent materials were adsorbed very strongly at the top of the column. The column was then washed with aqueous acetone (of increasing acetone concentrations up to 50%) again 0.1 *N* with respect to hydrochloric acid. The required compound was slowly eluted and the impurities were left in the column. The eluate was collected, reduced to a small bulk and neutralized with sodium hydroxide. It was then evaporated to dryness *in vacuo* and the residue crystallized (hot filtration) from water. After one further crystallization, the compound separated in small colorless plates (40 mg.).

Anal. Calcd. for $C_9H_{11}O_3N_5$: C, 45.6; H, 4.64; N, 29.5. Found: C, 45.4; H, 4.77; N, 29.7.

Chromatographic comparison of this material with Fl 4A is shown in Table II.

TABLE II

Solvent	Fl 4A	Synthetic material
n-Propanol, 1% ammonia (2:1)	0.46	0.46
n-Butanol, acetic acid, water (20:3:7)	.37	.37
3% Ammonium chloride	.75	.75
sec-Butanol, formic acid, water (8:2:5)	.65	.65

On treatment with sodium metaperiodate, this material consumed 1 mole/mole, liberated a mole of acetaldehyde and gave a pteridine exhibiting aldehydic properties. On oxidation with permanganate, however, and careful examination of the resulting carboxylic acid, a mixture of the 6- and 7-acids was obtained (these are readily distinguishable by their ultraviolet fluorescence in alkaline solution, the 6-carboxylic acid being sky blue, and the 7-, green[14]). The synthetic material was thus a mixture of 2-amino-4-hydroxy-6- and -7-(1′,2′-dihydroxypropyl)-pteridine. No method of separating these isomers has yet been found.

A product containing only a trace of the 7-isomer (as determined by oxidation) can be obtained by longer incubation of the rhamnotetrose with hydrazine, but there is a corresponding sharp drop in the yield.

Comparison of Fl 4B with Synthetic 2-Amino-4-hydroxypteridine.—The isolation of Fl 4B is described above and its absorption spectrum is recorded. The reactions which led to a direct comparison with synthetic 2-amino-4-hydroxypteridine are described in outline in the first part of this paper. The paper chromatographic comparison of the natural and synthetic materials is given in Table III.

TABLE III

Solvent	Fl 4B	2-Amino-4-hydroxypteridine
n-Propanol, 1% ammonia (2:1)	0.44	0.44
3% Ammonium chloride	.55	.55
sec-Butanol, formic acid, water (8:2:5)	.72	.72
Butanol, acetic acid, water (4:1:5)	.45	.45
5% Acetic acid	.66	.66

Deamination of Fl 4B.—Deamination was carried out by adding a solution of Fl 4B in concd. hydrochloric acid to a solution made up from equal volumes of sodium nitrite solution (20%) and glacial acetic acid, according to the pro-

cedure used for rhizopterin.[15] After evaporation of the reaction solution to dryness, the residue was taken up in water, and the pteridine in this solution purified by paper chromatography using dilute acetic acid as solvent. The material obtained in this way was rechromatographed in the solvents indicated in Table IV and compared with a sample of synthetic 2-amino-4-hydroxypteridine, deaminated and purified in the same way.

TABLE IV

Solvent	Fl 4B	Deamination product	2,4-Dihydroxypteridine
n-Propanol, 1% ammonia (2:1)	0.44	0.14	0.14
n-Butanol, acetic acid, water (4:1:5)	.37	.23	.23
3% Ammonia chloride	.55	.53	.53
5% Acetic acid	.66	.48	.48

The absorption spectrum of this deaminated material showed peaks at 255 and 360 mμ in 0.1 *N* sodium hydroxide and at 325 mμ in 0.1 *N* hydrochloric acid, again corresponding exactly to authentic 2,4-dihydroxypteridine.

Comparison of Fl 3 with Isoxanthopterin.—The absorption spectrum was identical with that of synthetic isoxanthopterin.[9] The R_F values of the two are compared in Table V.

TABLE V

Solvent	Fl 3	Isoxanthopterin
n-Propanol, 1% NH_3 (2:1)	0.20	0.20
n-Butanol, acetic acid, water (4:1:5)	.37	.37
3% Ammonium chloride	.37	.37
5% Acetic acid	.42	.42

Identification of Uric Acid.—A small amount of the "ultraviolet absorbing material"[3] (4 mg.) was isolated in the following way. In Part I of this series,[2] the purple compound, Fl 3, was described as being eluted from powdered cellulose columns, between the compounds described above and the yellow pigment. A concentrate of this band from a number of columns was made and the white solid so obtained was dissolved in dilute ammonia, treated with a large excess of charcoal, filtered, and the filtrate acidified. The resulting white solid was almost free of purple fluorescent material. Its absorption spectrum had a single maximum in both 0.1 *N* sodium hydroxide (at 295 mμ) and 0.1 *N* hydrochloric acid (at 284 mμ). These maxima are identical with those shown by uric acid. Paper chromatographic comparison with an authentic specimen (R_F in *n*-propanol, 1% ammonia, 0.35; in *n*-butanol, acetic acid, water, 0.26) and analysis confirmed this identity.

Anal. Calcd. for $C_5H_4N_4O_3$: N, 33.3. Found: N, 33.5.

PASADENA 4, CALIF.

(15) D. E. Wolf, *et al.* THIS JOURNAL, **69**, 2753 (1947).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Reaction of Hydratropoyl Chloride with Sodium Peroxide

BY FREDERICK D. GREENE

RECEIVED MAY 4, 1955

Hydratropoyl chloride (methylphenylacetyl chloride) has been treated with sodium peroxide under a variety of conditions. The products isolated, in the order of importance, were hydratropic acid, hydratropyl hydratropoate (both racemates), styrene, α-phenylethanol, *meso*- and *dl*-2,3-diphenylbutane and acetophenone. With optically active starting material, the alcohol portion of the ester was of *retained* configuration (91% retention, 9% inversion); the α-phenylethanol and the 2,3-diphenylbutane were partially active and of *retained* configuration. The results are discussed in terms of the intermediate formation of the diacyl peroxide of hydratropic acid and decomposition within solvent "cages."

In spite of the wealth of data on the decomposition of diacyl and diaroyl peroxides, little is known of the primary decomposition processes. On the grounds of the usual products that have been isolated (acids, esters, hydrocarbons) two principal mechanisms[1,2] have been advanced.

$$\text{RCOO—OOCR} \longrightarrow \text{RCOO}\cdot + CO_2 + \text{R}\cdot \quad \text{(A)}$$

$$\text{RCOO—OOCR} \longrightarrow 2\text{RCOO}\cdot \quad \text{(B)}$$

The decomposition of diaroyl peroxides has been interpreted in terms of mechanism B.[3,4] The greatly accelerated rate of decomposition of phenylacetyl peroxide has been attributed to the operation of mechanism A, in which the formation of a relatively stable radical, the benzyl radical, in the rate-determining step might result in a lower activation energy than that required of mechanism B.[5] The same authors suggested the possibility of cyclic decomposition processes, mechanism C, as another means of accounting for the rapid decomposition of bis-phenylacetyl peroxide. By this mechanism, the ester and/or the dimeric hydrocarbon are formed directly from the peroxide in one synchronous step.

$$\text{R—R} + 2CO_2 \longleftarrow \text{RCOO—OOCR} \longrightarrow \text{RCOOR} + CO_2 \quad \text{(C)}$$

In order to get information on the relative importance of these processes we have attempted to prepare an optically active peroxide.[6,7]

Results

The original intent was to prepare the diacyl peroxide of optically active hydratropic acid. All attempts to isolate this material failed; and, indeed, it was shown that even at −20° the rate of decomposition exceeded the rate of formation. Because of this limitation, the investigation involved the close scrutiny of the products derived from reaction of hydratropoyl chloride with sodium peroxide by four procedures.

(1) D. H. Hey and W. A. Waters, *Chem. Revs.*, **21**, 169 (1937).

(2) A. V. Tobolsky and R. B. Mesrobian, "Organic Peroxides," Interscience Publishers, Inc., New York, N. Y., 1954.

(3) G. S. Hammond, THIS JOURNAL, **72**, 3737 (1950); G. S. Hammond and L. M. Soffer, *ibid.*, **72**, 4711 (1950).

(4) C. G. Swain, W. H. Stockmayer and J. T. Clarke, *ibid.*, **72**, 5426 (1950).

(5) P. D. Bartlett and J. E. Leffler, *ibid.*, **72**, 3030 (1950). In addition to radical decomposition processes, phenylacetyl peroxide may decompose also by a general acid-catalyzed process.

(6) M. S. Kharasch, J. Kuderna and W. Nudenberg, *J. Org. Chem.*, **19**, 1283 (1954), recently have reported the decomposition of the optically active peroxide of α-methylbutyric acid in benzotrichloride solution at 75–80°. The ester, *sec*-butyl α-methylbutyrate, was isolated in 30–40% yield. Hydrolysis of the ester afforded optically active *sec*-butyl alcohol of 78% retention, 22% racemization.

(7) C. S. Marvel, R. L. Frank and E. Prill, THIS JOURNAL, **65**, 1647 (1943), reported the preparation of optically active *p*-*sec*-butylbenzoyl peroxide.