nucleated with β-isomaltose octaacetate and stirred with a magnetic stirrer for 24 hr. The crystalline material was filtered and was twice recrystallized from ethanol. Further crops of β-isomaltose octaacetate were obtained from the mother liquors and were recrystallized from ethanol; combined yield 64 g., m.p. 142–144°, $[\alpha]^{25}D$ +95° (*c* 4.6, chloroform).

β-Gentiobiose Octaacetate.—After the zone containing largely isomaltose had been removed from the carbon column with 3% ethanol, as indicated by a weak Benedict test, the developing solution was changed to 10% ethanol. When the Benedict test again became strongly positive the effluent was collected (20 liters) and evaporated to a sirup under reduced pressure. The sirup was dried by repeated addition of methanol and evaporation under reduced pressure; yield 120 g. This material was acetylated by the procedure described above; total yield 96 g. Pure material was obtained on recrystallization from ethanol; m.p. 191.5–192.5°, $[\alpha]^{27}D$ −4.6° (*c* 3.3, chloroform).

If it is desired to isolate only the gentiobiose from the reversion mixture, the dried unfermentable portion above may be acetylated directly and isolated. in a somewhat lower yield, as the β-octaacetate, as described by Berlin[9] for hydrol; yield 48 g. from 1600 g. of D-glucose, m.p. 190–192°, $[\alpha]^{25}D$ −5° (*c* 3.5, chloroform).

COLUMBUS 10, OHIO

[CONTRIBUTION FROM THE NUTRITION AND PHYSIOLOGY SECTION, RESEARCH DIVISION, AMERICAN CYANAMID COMPANY]

The Synthesis of Some 2-Amino-4-hydroxy-6-polyhydroxyalkyl-pteridines Which Are Active in Supporting the Growth of the Protozoön *Crithidia fasciculata*

BY E. L. PATTERSON, R. MILSTREY AND E. L. R. STOKSTAD

RECEIVED AUGUST 9, 1957

Four 2-amino-4-hydroxy-6-polyhydroxyalkyl-pteridines were prepared. Among these was an apparent correlation between the presence of two adjacent carbinols both having the L-configuration in the alkyl side chain and high potency in supporting the growth of the protozoön, *Crithidia fasciculata*.

A pteridine was isolated from normal human adult urine which was required for growth by the protozoön, *Crithidia fasciculata*. It was identified as 2-amino-4-hydroxy-6-[1,2-dihydroxypropyl-(L-erythro)]-pteridine and named biopterin.[1-3] The same compound identified except for optical configuration was one of the fluorescent substances extracted from *Drosophila melanogaster*.[4] In connection with the work on the proof of structure and synthesis of biopterin several 2-amino-4-hydroxy-6-polyhydroxyalkyl-pteridines have been prepared. This paper describes some of the properties of four of these compounds.

Experimental

The method of synthesis and the subsequent purification procedures outlined in the following example were essentially the same for all the pteridines. The aldoses having the proper configurations which were selected to prepare each of the pteridines are given in Table I along with some of the properties of the products.

The 2-Amino-4-hydroxy-6-[1,2,3-trihydroxypropyl-(L-erythro)]-pteridine (I).—One hundred and fifty g. (1 mole) of L-arabinose, 156 g. (1.7 moles) of anhydrous sodium acetate and 62.5 g. (1 mole) of boric acid were dissolved in 500 ml. of water. One hundred and twenty-six g. (0.89 mole) of 2,5,6-triamino-4-hydroxy pyrimidine (II) in 250 ml. of water prepared as a smooth slurry in a Waring blender was added slowly with good stirring. The *p*H of the pale yellow solution was 5.7. It was heated to 80° with agitation by means of a nitrogen stream, and 117 g. (2 moles) of 85% hydrazine hydrate was added. The *p*H was adjusted from 7.8 to 5.5 with about 100 ml. of acetic acid. The solution was heated to 95° for 1 hour and 15 minutes, and during this time it slowly turned dark brown in color, and a precipitate formed. The reaction mixture was cooled at 0° for a few hours and filtered. The weight of brown colored product was 64 g.

The crude precipitate was suspended in 2.5 l. of hot water which was then saturated with lime. The dark brown insoluble material was filtered off and washed twice with 2.5 l. of hot water and discarded. The filtrate and washings were combined and heated to 80° and stirred while a saturated zinc chloride solution was added slowly until the *p*H dropped to 8.6. The black insoluble material was filtered off and washed twice with 200 ml. of hot water and discarded. The combined filtrate and washings were bright yellow in color. The volume was reduced to 2 l. by distillation at reduced pressure.

A 6 × 16 in. chromatographic column of magnesol[5a] super cel[5b] (2:3 by weight) degassed by stirring in water was poured as a slurry and allowed to pack by gravity. The yellow pteridine solution was put on the column with the aid of 2 p.s.i. air pressure. The column was developed by gradient elution with 20 l. of water and 20 l. of 2 *N* ammonium hydroxide under 3 to 5 p.s.i. air pressure. The eluate was collected in fractions of 800 to 1000 ml., each representing a one-hour interval. The fractions saved were those in which the ratio of the ultraviolet absorption in 0.1 *N* NaOH at 253 mμ to that at 360 mμ fell in the range of 2.5 to 3. The combined selected fractions were reduced to near dryness by distillation at reduced pressure, and after standing overnight in 500 ml. of 3 *N* HCl a magnesol gel that had formed was removed by centrifuging, washed with about 25 ml. of 3 *N* HCl and discarded. The total solids in the combined centrifugate and wash weighed 20 g.

The sample from the magnesol column in 500 ml. of 3 *N* HCl was added to a 3 × 20 in. column of Dowex 50[5c] × 8 200–400 mesh in the hydrogen cycle which had been equilibrated with 3 *N* HCl. The column was developed by gradient elution with 4 l. of 3 *N* HCl and 4 l. of 6 *N* HCl. The flow rate was about 200 ml. per hour, and fractions were collected in two-hour intervals. The desired fractions were selected on the basis of ultraviolet absorption as before. The composite was reduced to dryness by distillation at reduced pressure, and the residue was dissolved in 200 ml. of water. The total solids in the sample weighed 7 g.

Upon the addition of lime to this solution a considerable quantity of material, mostly Dowex 50 resin, precipitated and was filtered off and discarded. The solution was heated to 80°, and saturated zinc chloride was added slowly with good stirring until the *p*H dropped to 8.6. The dark colored insoluble material was removed by filtration and discarded, and the yelllow filtrate was made acid with acetic acid. After standing at 4° overnight the pale yellow precipitate was filtered off and washed three times with acetone and air dried. The product weighed 2.7 g., and by microbiological assay with *C. fasciculata*,[6] it was 35% pure.

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(1) E. L. Patterson, H. P. Broquist, Alberta M. Albrecht, M. H. von Saltza and E. L. R. Stokstad, THIS JOURNAL, **77**, 3165 (1955).

(2) E. L. Patterson, M. H. von Saltza and E. L. R. Stokstad, *ibid.*, **78**, 5871 (1956).

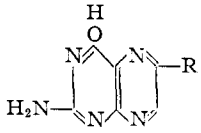
(3) E. L. Patterson, R. Milstrey and E. L. R. Stokstad, *ibid.*, **78**, 5868 (1956).

(4) H. S. Forrest and H. K. Mitchell, *ibid.*, **77**, 4865 (1955).

(5) (a) Magnesol industrial powdered, Westvaco Chemical Division, South Charleston, W. Va.; (b) Johns-Manville Corporation, New York, N. Y.; (c) The Dow Chemical Company, Midland, Michigan.

(6) For a description of the microbiological assay see Harry P. Broquist and Alberta M. Albrecht, *Proc. Soc. Expl. Biol. and Med.*, **89**, 178 (1955).

TABLE I



PROPERTIES OF CERTAIN 2-AMINO-4-HYDROXY-6-POLYHYDROXYALKYL-PTERIDINES

Reactants II + aldose	R		Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	$[\alpha]^{25}D^{a}$	Bioactivity[b]
L-Arabinose	—CH(OH)—CH(OH)—CH$_2$OH (L-erythro)	I	$C_9H_{11}N_5O_4$	42.69	42.81	4.35	4.77	27.67	27.41	−41.0	0.05
D-Ribose	—CH(OH)—CH(OH)—CH$_2$OH (D-erythro)	III	$C_9H_{11}N_5O_4$	42.69	42.83	4.35	4.36	27.67	27.65	+39.2	35
D-Xylose	—CH(OH)—CH(OH)—CH$_2$OH (D-threo)	IV	$C_9H_{11}N_5O_4$	42.69	42.96	4.35	4.68	27.67	27.80	−105	500
L-Rhamnose	—CH(OH)—CH(OH)—CH(OH)—CH$_3$ (L-arabino)	V	$C_{10}H_{13}N_5O_4$	44.94	44.27	4.87	5.37	26.21	26.12	+96.3	0.05

[a] 3 to 5 mg./ml. in 0.1 *N* NaOH. [b] mγ/ml. for 1/2 maximum growth of *C. fasciculata*.

The sample was recrystallized three times from boiling 20% acetic acid in water at a concentration of 4 mg. per ml. The pure material came out as pale yellow triclinic crystals in rosettes; decomposed without melting above 250°. The weight after the final crystallization was 0.7 g.

Using the growth of *C. fasciculata* as an assay, the yield of the desired products in the reaction of the aldoses with II was 1–2%, and the final recovery of the pure products was 0.3–0.5% calculated from the starting aldose.

By paper chromatography in the system 5% cyclohexylamine in *n*-butanol saturated with water, the permanganate oxidation product of each of the four pteridines was exclusively 2-amino-4-hydroxypteridine-6-carboxylic acid.[2,4]

Discussion

The reaction of II with sugars gives mixtures of 2-amino-4-hydroxy-6- and 7-polyhydroxyalkyl-pteridines (6-isomer and 7-isomer)[4,7–10] with the 7-isomer predominating. In the presence of certain oxidizing agents in stoichiometric amounts, particularly hydrazine, the synthesis of the 6-isomer is favored starting with D-glucose,[8,9] D-fructose,[9] L-sorbose,[8] *p*-tolyl-D-isoglucosamine[10] and D-glucosone.[9] The reaction of L-arabinose, *p*-tolyl-L-isoarabinosamine[11] and L-arabinosone[12] with II was studied in this investigation under these same conditions in an attempt to increase the yield of I. Using the growth response of *C. fasciculata* to the crude reaction mixtures as a measure of the amount of I formed, the yield was never increased above 2%, which is in agreement with previous indications that no marked directive effect toward the synthesis of the 6-isomer was observed with the pentoses.[8]

In some of the first preparations samples in various stages of the purification scheme, in particular the fractions off the magnesol and ion-exchange columns, were assayed for their growth stimulation of *C. fasciculata* and for their pteridine content by fluorescence in 0.1 *N* sodium borate *p*H 9 and by ultraviolet absorption in 0.1 *N* NaOH at 253 and 360 mμ. The correlation between the bioassay and the fluorescence was often poor, apparently because impurities present in substantial amounts exhibited a fluorescence. However, there was general agreement between the bioassay and the ratio *E* 253 mμ/*E* 360 mμ in 0.1 *N* NaOH in the range of 2.5 to 3.0. Therefore, for routine runs this range in the ratio of the ultraviolet absorption maxima was used as a guide to the selection of the desired fractions because it was more convenient than the bioassay.

The optical rotation values of I, III and IV (Table I) are in disagreement with those for the same compounds reported by Karrer, Schwyzer, Erden and Siegwart.[7] An explanation of the difference may be that the samples made by the earlier investigators were mixtures of the 6- and 7-isomers with the 7-isomers predominating. To test this hypothesis, L-arabinose was treated with II and the product purified according to the procedures of Karrer, Schwyzer, Erden and Siegwart.

(7) P. Karrer, R. Schwyzer, B. Erden and A. Siegwart, *Helv. Chim. Acta*, **30**, 1031 (1947).

(8) H. G. Petering and J. A. Schmitt, THIS JOURNAL, **71**, 3977 (1949).

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

(10) F. Weygand, A. Wacker, V. Schmied-Kowarzik, *Chem. Ber.*, **82**, 25 (1949).

(11) Prepared according to the procedure of F. Weygand, *ibid.*, **73**, 1259 (1940). The product was not isolated.

(12) Prepared according to the procedure of E. Fischer and E. F. Armstrong, *ibid.*, **35**, 3141 (1902). The product was not isolated.

This material had an optical rotation of $[\alpha]^{24}D$ +33° (*c* 0.5, 0.1 *N* NaOH) compared to the Swiss authors' value of $[\alpha]^{20}D$ +29.3 in 0.1 *N* NaOH. The amount of the 6-isomer in this material was less than 1% measured by the growth stimulation of *C. fasciculata*, and after oxidation with permanganate the only major product observed by paper chromatography was 2-amino-4-hydroxypteridine-7-carboxylic acid.[2,4]

It is of interest to note the relationship between the optical configuration about the asymmetric carbon atoms in the polyhydroxyalkyl side chain of these pteridines and their activity in supporting the growth of *C. fasciculata*. Only the compounds containing two adjacent carbon atoms with the L-configuration are highly active—namely, biopterin,[3] I and V. It is not required that one of these carbon atoms be attached to the pyrazine ring, and the presence of other carbinols, either primary or secondary, is without appreciable effect. The biological significance of this configurational specificity is not understood at this time.

Acknowledgment.—The authors are indebted to Mr. L. Brancone and staff for the microanalyses.

PEARL RIVER, N. Y.

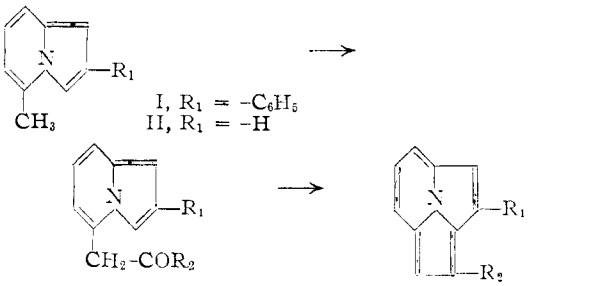
COMMUNICATIONS TO THE EDITOR

CYCLAZINES. THE SYNTHESIS OF A NEW CLASS OF AROMATIC COMPOUNDS[1]

Sir:

We wish to report the synthesis of a new class of aromatic compounds having the general structure shown by IX, for which we propose the trivial name, cycl[3,2,2]azine.[2] The general procedure employed in these syntheses is illustrated below.

When 2-phenyl-5-methylpyrrocoline, I (m.p. 83–83.5°. Found: C, 87.16; H, 6.65), prepared by the Chichibabin procedure,[3] was treated with *n*-



butyllithium followed by N,N-dimethylbenzamide, ketone III (m.p. 125–127°. Found: C, 84.92; H, 5.83) resulted. Heating III in glacial acetic acid gave VII (yellow crystals, m.p. 143.5–144°. Found: C, 90.09; H, 5.22; N, 4.79; mol. wt., 295). Similarly, substitution of dimethyl formamide for dimethylbenzamide in the reaction sequence gave the aldehyde IV, which was cyclized directly to give VIII (yellow crystals, m.p. 98–99°. Found: C, 88.12; H, 5.27). As evidence for the proposed structures, it was found that treatment of 5-methylpyrrocoline,[4] II (b.p. 124° at 34 mm. Found: C, 82.00, H, 7.15, N, 10.93) with *n*-butyllithium and dimethylbenzamide gave V (m.p. 111–112°. Found: C, 82.15; H, 5.73) which cyclized to give VIII, identical in all respects with the sample previously described. Finally, repetition of the reaction sequence using 5-methylpyrrocoline and dimethylformamide gave VI, which on cyclization yielded the parent cycl[3,2,2]azine, IX (yellow crystals, m.p. 65–66°. Found: C, 84.93; H, 5.30; N, 9.87).

In contrast to the behavior of pyrrocolines, the cyclazines show unusual stability toward air, light and heat. Also, they show a complete lack of basicity; the ultraviolet absorption spectrum, characteristic of the system, is unaffected by added acid. These observations are in general accord with the predictions of simple molecular orbital theory.[5]

(1) Aided in part by the Office of Ordnance Research, Army Ordnance Contract No. DA-30-115-ORD-723.

(2) In this proposal of nomenclature, the word cyclazine would be reserved for the general case of a conjugate, unsaturated cycle held planar by three covalent bonds to an internal nitrogen atom. The various possible cyclazines which then arise through having cycles of different size or different points of attachment to nitrogen can be distinguished by placing in brackets numerals which correspond to the number of atoms on the cycle between points of fusion, *i.e.*, IX becomes cycl[3,2,2]azine.

(3) A. E. Chichibabin, *Ber.*, **60**, 1607 (1927).

(4) The preparation of 5-methylpyrrocoline, which has been accomplished by two independent routes, will be described in the full publication.

(5) These calculations will be presented in the full publication.

(6) National Science Foundation Predoctoral Fellow, 1956–1958.

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V. BOEKELHEIDE
R. J. WINDGASSEN[6]

RECEIVED MARCH 21, 1958

A ONE-STEP TRANSFORMATION OF ACETOPHENONE INTO BENZALDEHYDE[1]

Sir:

By means of a new method, acetophenone has been transformed in one step into benzaldehyde. There does not appear to be another known method for a one-step degradation of an acyl substituent

(1) Financial assistance under a National Institutes of Health Grant No. H-2295(c) and Contract No. DA-01-009-ORD-428 with Office of Ordnance Research, U. S. Army, is gratefully acknowledged.