THE CHEMISTRY OF THE PTERIDINES

MARSHALL GATES

Department of Chemistry, Bryn Mawr College, Bryn Mawr, Pennsylvania

Received February 28, 1947

CONTENTS

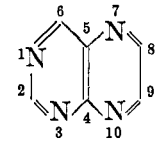
I. Introduction

Knowledge of the pteridines[1] originated in the 1890's when Hopkins (30, 31, 32) published the results of his investigations on the wing pigments of the common English brimstone butterfly and the white cabbage butterfly. At about the same time, synthetic work by Kühling resulted in the first laboratory preparation of a substance with this nucleus (48). The relationship between these efforts was not clearly realized until 1940 (73, 74, 102), when the wing pigments, now known as "pterins" (88, 108), of the butterflies investigated by Hopkins were shown by Wieland and his collaborators to be derived from the pteridine ring system.

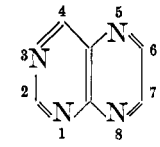
The recognition that one of the pterins, xanthopterin, occurs widely in animal organisms and bears some relationship, the nature of which is still obscure, to hematopoiesis, and the recent demonstration that folic acid, one of the newest members of the vitamin B complex and a substance of considerable therapeutic importance, contains this nucleus, have resulted in greatly increased interest in this class.

The pteridine ring system is composed of fused pyrimidine and pyrazine rings, illustrated in the accompanying formulas:

[1] The name "pteridine" appears first to have been applied to this ring system by Weygand (101; see also 81), but his publication has not been available to the author. The names alloxazine (48) (no longer suitable in the light of later work on alloxazines and isoalloxazines (49)), azine-purine (79), and lumazine (49) have also been proposed for compounds of this nucleus carrying two hydroxyl groups in the pyrimidine ring.



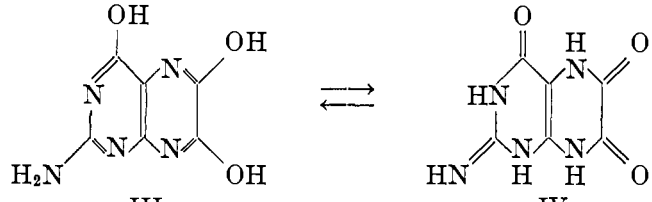
I



II

Its systematic name, according to the *Ring Index* (61), is pyrimido[4,5-*b*]pyrazine. The numbering shown in formula I, related to that used with the purines, is used exclusively in the extensive publications from German laboratories on the pterins, and has been adopted throughout this review, although the alternative numbering shown in II is preferred in the *Ring Index* and has been used in important American papers (2).

Most of the known pteridine derivatives have several hydroxyl or amino substituents and therefore possess manifold tautomeric possibilities, two of which are illustrated for leucopterin:



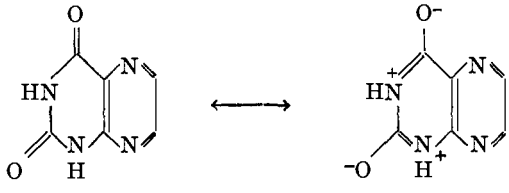
III IV

A choice between such tautomers cannot be made on the available evidence; in this article the "enolic" form with a completely aromatized system of double bonds, as illustrated in III, has arbitrarily been used in all systems where complete aromaticity is possible.[2]

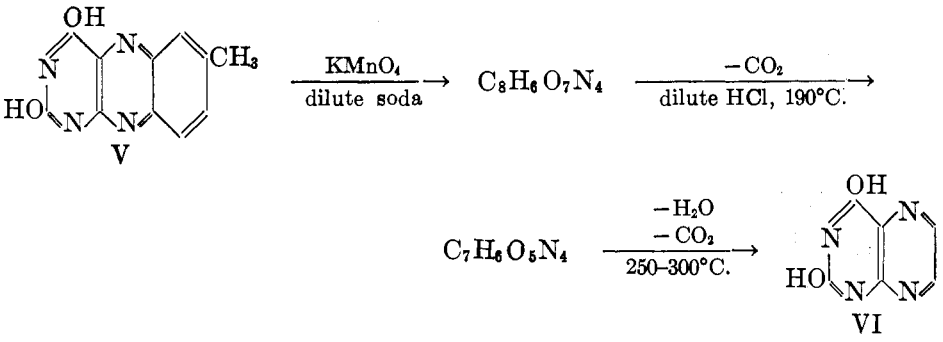
II. Synthesis of the Pteridine Ring System

A number of syntheses leading to derivatives of the pteridine nucleus have been described. The earliest successful synthesis of this ring system was carried out in 1895 by Kühling (48) who, having ascertained that the reaction of alloxan with aliphatic 1,2-diamines fails (47), was able to degrade "tolualloxazine" (V) by oxidative methods to 2,6-dihydroxypteridine (VI).

[2] The low solubilities in non-polar solvents and the high melting points exhibited almost without exception by these substances might be taken to indicate that the keto form, in which contributions from betaine-like structures are possible, predominates:

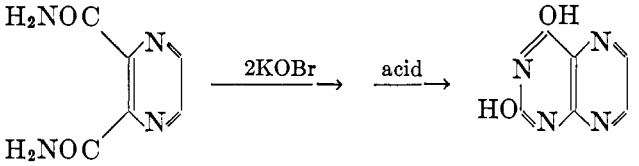


m.p. > 350°C. (49)



Kühling noted the high stability of the ring system toward both acids and alkalies.

Another early synthesis yielding the same compound and, like the above, of only historical interest, was accomplished by Gabriel and Sonn (23), who obtained the potassium salt of 2,6-dihydroxypteridine by the action of 2 moles of potassium hypobromite on the diamide of 2,3-pyrazinedicarboxylic acid.

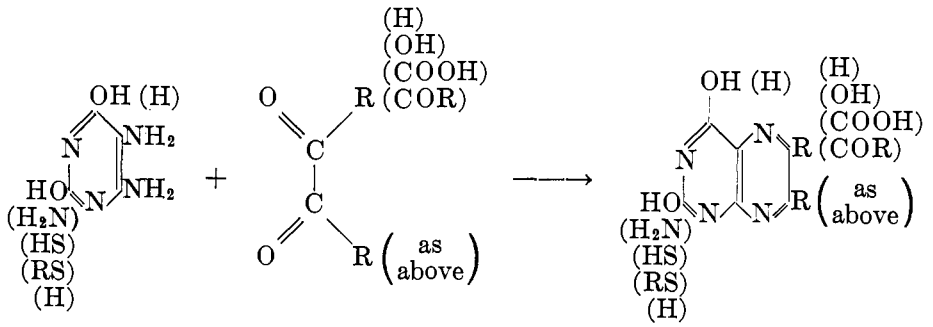


The smooth condensation of 1,2-dicarbonyl compounds with 4,5-diaminopyrimidines to yield derivatives of the pteridine nucleus was first systematically studied by Sachs and Meyerheim (79), although the reaction had previously been put to use for the characterization of diaminopyrimidines by Traube and his students (79) and by Isay (36). Sachs and Meyerheim showed that 3-methyl- and 1,3-dimethyl-2,6-dihydroxy-4,5-diaminopyrimidines condense smoothly with such varied dicarbonyl compounds as diacetyl, triketopentane, pyruvic and mesoxalic acids, alloxan, and also with ethyl dichloroacetate. 2,4,5-Triamino-6-hydroxypyrimidine, which was later to be of great value in the synthesis of the pterins, was used in one example. No attempt was made by Sachs and Meyerheim to assign structures to products resulting from unsymmetrical dicarbonyl compounds.

This reaction was also useful to Kuhn and Cook (49) and to Ganapati (24) in the synthesis of pteridines related to the flavines. Many examples of the condensation of 2,6-dihydroxy-4,5-diaminopyrimidine with 1,2-dicarbonyl compounds are given by them. Glyoxal, methylglyoxal, phenylglyoxal, diacetyl, β-naphthoquinone, phenanthrenequinone, benzil, 3,4,3′,4′-bis(methylenedioxy)benzil, dihydroxytartaric acid, camphorquinone, isatin, and alloxan were all used successfully. The reaction fails with *o*-benzoquinone (49), and Kuhn has pointed out that for the synthesis of alloxazines, only the condensation of alloxan with *o*-phenylenediamine is useful, in contrast to the pteridine series, where the analogous condensation between alloxan and aliphatic 1,2-diamines

is unsuccessful (47, 110). Again, the structures of products from unsymmetrical dicarbonyl compounds were not ascertained.

The general reaction may be represented by the following equation:



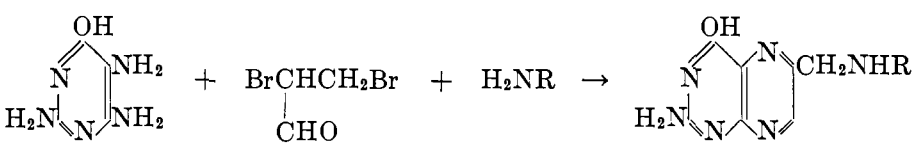
or tautomers with the ring nitrogens alkylated

or tautomers with the pyrimidine ring nitrogens alkylated

Many applications of this reaction and its modifications have been made recently in the pterin field, where the complicated and confusing structural picture resulting from degradative work was greatly clarified by Purrmann's synthesis of leucopterin in 1940 (73). More detailed accounts of syntheses in this field will be given later, and it is only necessary to say here that successful application of the above reaction, using (*1*) 2,6-dihydroxy-4,5-diaminopyrimidine, 2,4,5-triamino-6-hydroxypyrimidine, 2,4,5-triaminopyrimidine, and 2-hydroxy-4,5,6-triaminopyrimidine as diamine components, and (*2*) oxalic acid, ethyl mesoxalate, dichloroacetic acid, glyoxal, and glyoxylic acid as dicarbonyl components, has been achieved (2, 42, 73, 74, 75, 76, 77, 87, 104, 110).

Recently Polonovski and his coworkers (68, 70) and Wieland and Liebig (104) have extended the synthesis to 2-thiol-4,5-diaminopyrimidines and their thio ethers.

A further variation of this general synthesis consists in the use of α-halogen carbonyl compounds with 4,5-diaminopyrimidines. In this case, a dihydro compound is formed as an intermediate but is oxidized to the pteridine during the reaction (2). The important synthesis of the *Lactobacillus casei* liver factor, which will be discussed later, was of this type, and made use of 2,4,5-triamino-6-hydroxypyrimidine, α, β-dibromopropionaldehyde, and *p*-aminobenzoyl-*l*(+)-glutamic acid (2).



Note added in proof: Karrer and his coworkers (39a) have recently described the synthesis of pteridines carrying hydroxylated side chains in the 8(or 9)-position by the condensation of 2,4,5-triamino-6-oxypyrimidine with both aldoses and ketoses.

III. Physical Properties of the Pteridines

The simple pteridine nucleus is unknown, but those derivatives which have been studied, containing functional groups (hydroxyl, amino, or sulfhydryl) in the 2- and 6-positions (pyrimidine ring) and functional groups or alkyl and aryl groups in the 8- and 9-positions (pyrazine ring), are virtually all very high-melting substances of sparing solubility in the ordinary organic solvents and in cold water, although polyhydroxy compounds such as ethylene glycol and glycerol appear to have some solvent power (33, 82).

The ultraviolet and visible light absorption of the pteridines has been thoroughly studied (7, 13, 22, 39, 49, 71, 82) and strongly resembles that of the flavines and alloxazines (7, 50). Simple derivatives carrying functional substituents only in the 2- and 6-positions are yellow; those substituted in the pyrazine ring may be yellow or colorless according to the number and position of the substituents.

One of the most striking characteristics of the pteridines is their strong fluorescence, which is similar to that of the flavines (50). Even the simple members exhibit this tendency so strikingly that Kuhn proposed the generic name "lumazine" for 2,6-dihydroxypteridines (49). The nuance of the fluorescence varies from compound to compound, and in any one compound it may vary characteristically with pH. For example, in strong solutions of mineral acids xanthopterin shows a strong red fluorescence under ultraviolet light (3, 40), while at pH's from 7 to 11 the fluorescence is sky blue (40). Sensitive microchemical tests based on fluorescence and adsorption behavior have been developed for the recognition of small amounts of pterins in insects and in other biological materials (3, 17, 40, 104; see also 39, 55, 62, 66, 69).

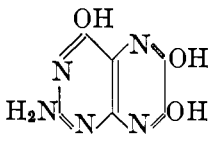
Polonovski and his coworkers (68, 70, 71), during synthetic studies on sulfur-containing pteridines, have observed that 2-thiol-6-hydroxypteridines do not fluoresce, whereas the corresponding 2-ethylthio ethers possess strong blue or green fluorescence similar to that of the 2,6-dihydroxypteridines. Moreover, the 2-thiol compounds inhibit the fluorescence of the corresponding thio ethers.

The lack in the pterins of the usual criteria for identity has led to rather widespread use of DeBye–Scherrer diagrams for this purpose by Schöpf (83) and by Wieland and his collaborators (106; and later papers); such diagrams are available for a number of natural and synthetic pteridine derivatives. The x-ray diagram of leucopterin has also been studied from the structural point of view without conclusive results (56).

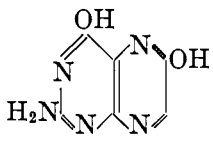
IV. Chemical Properties of the Pteridines

A. General Chemical Properties

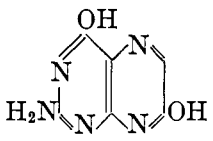
Much of our knowledge of the general chemistry of the pteridines has resulted from structural studies carried out on the naturally occurring pterins by European workers, and the two most important pterins, leucopterin (VII) and xanthopterin (VIII), together with isoxanthopterin (IX) will figure prominently in the following discussion.



VII
Leucopterin



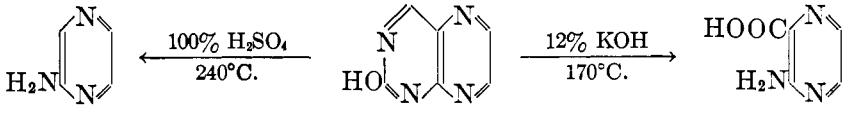
VIII
Xanthopterin



IX
Isoxanthopterin

Virtually all the pteridines have well-developed acidic properties and are soluble in both alkalies and ammonia (13, 23, 24, 48, 49, 105, 108). Quantitative data are available for only two compounds, 2-amino-6-hydroxypteridine, whose pK_a is 8.0, and 2-amino-6-hydroxy-8-pteridinecarboxylic acid, whose pK_a's are 3.9 and 7.7, respectively, for the first and second dissociations (2). The presence of an amino group in the 2-position confers basic properties as well, although the substituents present in the pyrazine ring and their positions markedly affect the degree of basicity. For example, leucopterin (VII), with two hydroxyl groups in the 8- and 9-positions, is nearly devoid of basic properties, resembling uric acid in this respect (105). It does form a yellow sulfate in fairly concentrated sulfuric acid (104). On the other hand, xanthopterin (VIII), carrying a hydroxyl in the 8-position, has rather pronounced basic properties. Isoxanthopterin (IX), differing from xanthopterin only in the position of the hydroxyl in the pyrazine ring, is significantly less basic than xanthopterin (75) and is also colorless, as is leucopterin, whereas xanthopterin is yellow. Purrmann has discussed the theoretical basis for these differences in terms of inductive effects (75).

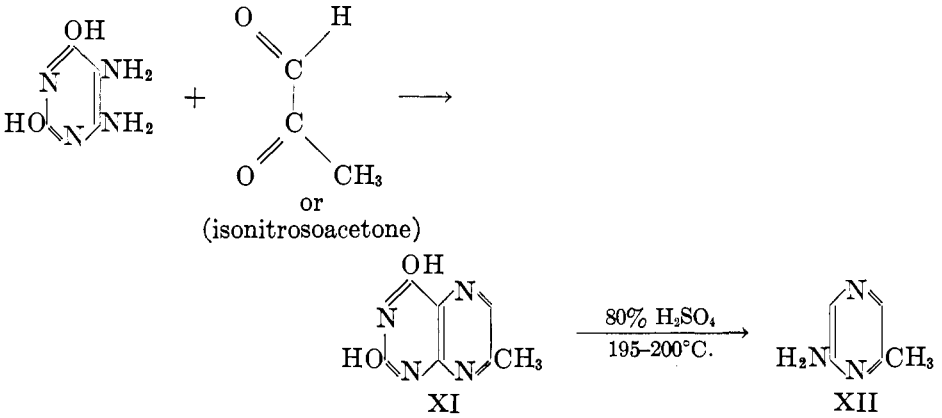
Simple pteridine derivatives carrying hydroxyl groups only on the pyrimidine ring are very stable towards hydrolysis (48), but under appropriate conditions degradation of the pyrimidine ring occurs with either acids or bases (100). For example, 2,6-dihydroxypteridine (X), heated with 100 per cent sulfuric acid to 240°C., gives 2-aminopyrazine in high yield. Homologs give lower yields however, and optimum results with homologs are obtained with somewhat more dilute acid and lower temperatures. The same compound is degraded by heating with 2 to 3 equivalents of 12 per cent alkali for 2 hr. at 170°C. to 2-aminopyrazine-3-carboxylic acid in 93.5 per cent yield. With stronger alkali or longer heating, some of the corresponding 2-hydroxy compound is produced, although higher homologs yield only the amino compound (19, 100).



X
2,6-Dihydroxypteridine

These degradations were developed during a search for better methods for preparing 2-aminopyrazines and in addition to the above example have been applied to 8- or 9-methyl-, 8 or 9-phenyl-, 8,9-dimethyl- and 8,9-diphenyl-2,6-dihy-

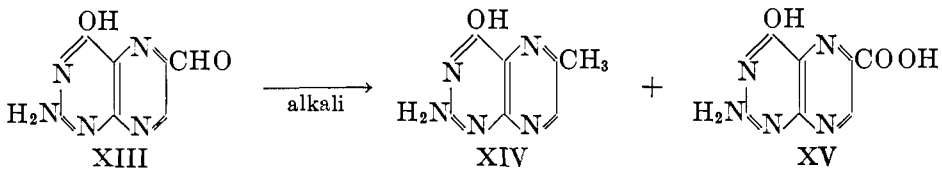
droxypteridines. From the monomethyl compound (XI) an aminomethylpyrazine not identical with 2-amino-5-methylpyrazine and therefore presumably 2-amino-6-methylpyrazine (XII) was obtained. This implies that the monomethyl compound is 2,6-dihydroxy-9-methylpteridine and that the synthesis used in its preparation proceeds as follows (100) (see page 65):



XI XII

8-Alkyl-2-amino-6-hydroxypteridines are converted to the corresponding carboxylic acid on oxidation with hot alkaline permanganate (2). The acid may be decarboxylated by heating to 300°C. to yield the parent 2-amino-6-hydroxypteridine (2).

An interesting dismutation is undergone by 2-amino-6-hydroxy-8-pteridinealdehyde (XIII) on standing in alkaline solution in the absence of oxygen. Under these conditions, the aldehyde is converted into a mixture of the 8-methyl derivative (XIV) and the 8-carboxylic acid (XV) (2).

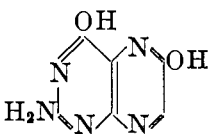


XIII XIV XV

2,6-Dihydroxy-8,9-diphenylpteridine reacts with diazomethane to yield a dimethyl derivative of unknown structure (24).

B. XANTHOPTERIN, ISOXANTHOPTERIN, AND LEUCOPTERIN

These three naturally occurring substances are by far the best-characterized pteridine derivatives. In spite of their structural similarity, they differ strikingly in chemical behavior.



XVI

Xanthopterin

Xanthopterin (XVI) is a yellow solid melting above 400°C., which is obtained crystalline with difficulty (82, 108). It is very slightly soluble in the ordinary organic solvents and in cold water, although hot ethylene glycol (33) and warm glycerol (82) dissolve it, and it is freely soluble in hot water (29). It is amphoteric, behaving both as a base and as a weak dibasic acid, and its beautifully crystalline barium salt (called "β-xanthopterin barium salt" when prepared under controlled conditions) (82) is particularly suitable for purification and characterization (108). Partial hydrolysis of the barium salt occurs on crystallization from water, and it must be recrystallized from half-saturated barium hydroxide (82). The sodium and potassium salts can be obtained crystalline but in poor yield, and amorphous silver, gold, lead, and palladium salts are known (42, 82).

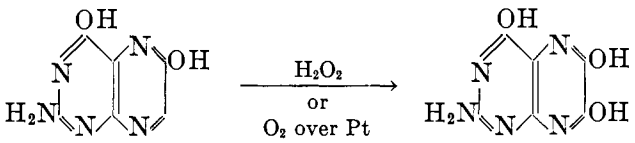
In contrast to leucopterin, the basic properties of xanthopterin are well developed, and it is readily soluble in dilute mineral acids from which it may be precipitated by sodium acetate (108). It appears to be nearly completely ionized in anhydrous formic acid, since its molecular weight in this solvent is about one-half of the nominal value (85).

With sulfurous acid, either xanthopterin or its barium salt forms a colorless crystalline adduct from which it may be regenerated by either acid or alkali (106). It gives a murexide test (29, 30, 108) but is more stable to dilute nitric acid than is uric acid (108).

Xanthopterin, like xanthine, couples with diazotized 2,5-dichloroaniline (82). Similarly, the substance prepared by Sachs and Meyerheim from 4,5-diamino-2,6-dihydroxy-1,3-dimethylpyrimidine and dichloroacetic ester, which presumably has the same configuration in the pyrazine ring as xanthopterin, couples with diazotized nitroaniline (79), and the ability to couple may be a general characteristic of 8-hydroxypteridines.

Nitrous acid readily attacks xanthopterin but unlike leucopterin, which yields the corresponding 2-desamino compound, the whole molecule is disrupted. Neither sodium nitrite and sulfuric acid nor nitrosylsulfuric acid in concentrated sulfuric acid lead to desaminoxanthopterin (85), which can, however, be prepared synthetically. It is colorless and less basic than xanthopterin (104). In the Van Slyke determination, xanthopterin loses 34 per cent of its nitrogen as gaseous nitrogen (41).

A variety of oxidizing agents attack xanthopterin readily. Its behavior toward hydrogen peroxide is particularly interesting. With perhydrol in acetic acid at room temperature, a colorless peroxide is produced which reverts to xanthopterin on treatment with alkali or on heating in aqueous suspension (106). The peroxide will oxidize iodide ion to iodine (106), and both it and xanthopterin on standing for several days at room temperature with an excess of perhydrol are converted into leucopterin (106, 107). On heating with excess perhydrol the peroxide yields melanurenic acid (106), and it has also been possible to isolate 2-iminooxonic acid in low yield as a result of the action of hydrogen peroxide on xanthopterin (107). The conversion of xanthopterin to leucopterin may also be carried out in 63 per cent yield by the use of molecular oxygen over platinum (107).



Hopkins' early observation that crude xanthopterin could be oxidized to "uric acid" by warm dilute nitric acid was considered by Schöpf (83) to have been merely the result of solution of the xanthopterin present as its nitrate (colorless). This would leave the contaminating non-basic leucopterin behind. That this would result with xanthopterin containing leucopterin as an impurity cannot be denied, but in view of the ease with which xanthopterin can be oxidized to leucopterin, Hopkins' observation perhaps merits reconsideration.

Xanthopterin is rapidly attacked by sodium chlorate in hydrochloric acid solutions at 100°C. with the formation, through transient intermediates, of oxalic acid, glyoxylic acid, guanidine, and urea, but no alloxan. At 80°C., oxalylguanidine can be isolated (85). 2,4,5-Triamino-6-hydroxypyrimidine yields oxalylguanidine and oxalic acid under the same conditions, and the isolation of these substances was taken by Schöpf to indicate the presence in xanthopterin of a 2-aminopyrimidine ring (85).

Ozone rapidly attacks xanthopterin, yielding oxalic acid, oxalylguanidine, and guanidine (85).

A variety of reducing agents readily transform xanthopterin into a colorless leuco compound, dihydroxanthopterin. Zinc dust in either acid or alkali (108), fuming hydriodic acid (110), either sodium sulfite or sodium hydrosulfite in neutral solutions (40), glutathione in neutral solutions (40), and hydrogen sulfide in neutral or alkaline solutions (40) will bring about this reduction. The reaction is at least partially reversible, and dihydroxanthopterin, prepared either by reduction of leucopterin with sodium amalgam (92) or by decarboxylation of dihydroxanthopterincarboxylic acid (75), can be converted to xanthopterin in 83 per cent yield by molecular oxygen over platinum (75) or in good but unspecified yield by oxidation with alkaline silver solutions (92). According to Koschara (41), when hydrogen sulfide is used to reduce xanthopterin, a sulfur-containing substance is formed in part, and catalytic reduction is not entirely reversible, since when dihydroxanthopterin solutions prepared in this way are shaken with air and then again reduced catalytically, only about 50 per cent of the original hydrogen is taken up.

Dihydroxanthopterin and its sulfate can both be obtained crystalline (75, 110). It reduces hypobromite, Chloramine T, quinone, methylene blue, and 2,6-dichlorophenolindophenol, in addition to the reagents mentioned above (92).

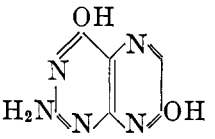
An interesting difference in the ease of decarboxylation between xanthopterincarboxylic acid and its colorless dihydro derivative has been observed. The former cannot be decarboxylated, but the latter readily yields dihydroxanthopterin by loss of carbon dioxide (75).

Xanthopterin is stable towards dilute hydrochloric acid and may be concentrated with it many times (108). Energetic acid hydrolysis of xanthopterin or

its barium salt produces glycine, isolated as hippuric acid, in 40–45 per cent yield. The yield may be raised to 56–59 per cent by preliminary reduction to dihydroxanthopterin. Under the same conditions, 2,4,5-triamino-6-hydroxypyrimidine, guanine, and uric acid give hippuric acid in 58–67 per cent yield (84).

Short hydrolysis with strong potassium hydroxide does not affect xanthopterin, although on continued hydrolysis with barium hydroxide a mole of ammonia is slowly lost (82).

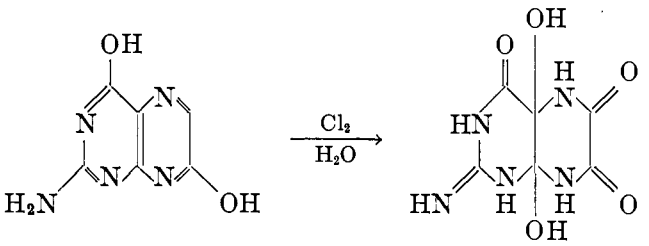
Isoxanthopterin (XVII) (8-desoxyleucopterin) differs strikingly from xan-



XVII

Isoxanthopterin

thopterin. It is colorless, significantly less basic, and dissolves in fuming hydriodic acid with the formation of a dihydro compound and the precipitation of 2 equivalents of iodine. On dilution of the solution, the iodine is reduced and isoxanthopterin reprecipitates (75, 110). Xanthopterin likewise is reduced by fuming hydriodic acid, but the dihydro compound is not immediately reoxidized on dilution.[3] A further difference is shown by the corresponding



XVIII

Leucopterin glycol

[3] The extraordinary behavior of isoxanthopterin toward fuming hydriodic acid may be a consequence of its low basicity. Presumably the following equilibria are set up:

$$\text{Isoxanthopterin} + \text{HI} \rightleftarrows \underset{\text{hydroiodide}}{\text{Isoxanthopterin}} \underset{I_2}{\overset{2HI}{\rightleftarrows}} \underset{\text{hydroiodide}}{\text{Dihydroisoxanthopterin}}$$

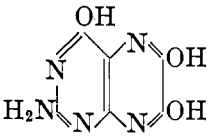
$$\text{Xanthopterin} + \text{HI} \rightleftarrows \underset{\text{hydroiodide}}{\text{Xanthopterin}} \underset{I_2}{\overset{2HI}{\rightleftarrows}} \underset{\text{hydroiodide}}{\text{Dihydroisoxanthopterin}}$$

In fuming hydriodic acid, both substances dissolve as hydroiodides, and both series of equilibria are displaced toward the right, whereas in dilute hydriodic acid, isoxanthopterin, being much less basic, precipitates as the free base, and the equilibrium in this case is shifted toward the left, even if the values of the equilibrium constants for the two redox reactions are similar.

carboxylic acids. Isoxanthopterincarboxylic acid, in contrast to xanthopterincarboxylic acid, is readily decarboxylated in high yield by heating to 260°C. (75).

Isoxanthopterin reduces Tollens' reagent and phosphotungstic acid, gives a murexide test, and forms yellow sodium and silver salts (105). In contrast to xanthopterin, it cannot be converted to leucopterin by oxidation, although on treatment with chlorine water, leucopterin glycol (XVIII) is formed. Oxalylguanidine may also be isolated from this reaction (103).

Leucopterin (XIX) is a colorless crystalline solid of high melting point. It is



XIX

distinctly acidic, dissolving readily in dilute alkalies and ammonia (105). Its yellow monopotassium salt is particularly characteristic and serves for purification (87). In contrast to uric acid, which reduces silver salts to metallic silver, leucopterin yields a bright yellow silver salt with silver nitrate (88). It is, however, superficially very similar to uric acid, and was mistaken for this substance by early workers (31, 111).

It exhibits only the feeblest basic properties (105), although it is possible to prepare a yellow sulfate in fairly concentrated sulfuric acid (104). It is insoluble in fuming hydriodic acid and may be separated from isoxanthopterin by making use of this property (110).

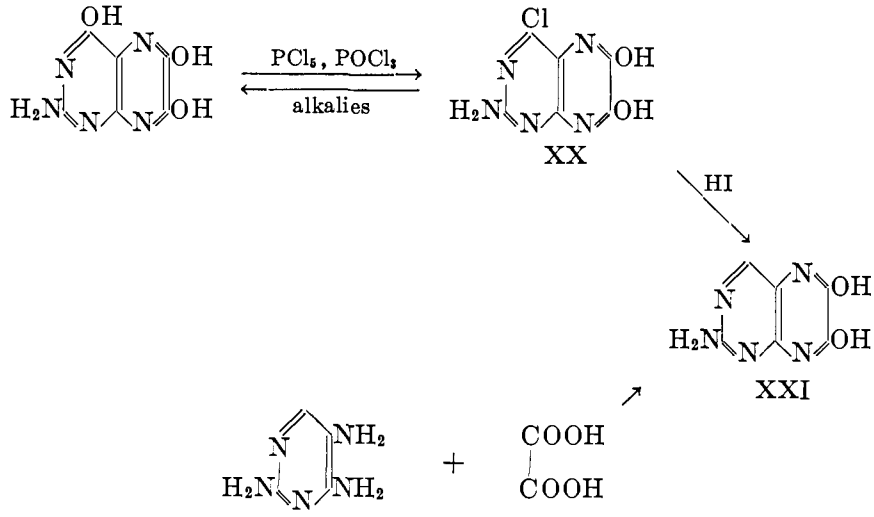
Leucopterin gives the murexide test, yielding a somewhat bluer tone than uric acid (88, 105).

A monoacetyl derivative of unknown structure is produced by the action of acetic anhydride and sulfuric acid on leucopterin. The acetylation does not proceed in pyridine or with acetic anhydride alone, and benzoylation fails both in pyridine and in sulfuric acid (105).

Leucopterin is desaminated by treatment with sodium nitrite in fairly concentrated sulfuric acid to give 2,6,8,9-tetrahydroxypteridine (desaminoleucopterin)[4](105). Isoleucopterin (6-amino-2,8,9-trihydroxypteridine) is not desaminated by nitrous acid (104) nor is isoguanine, a purine in which a similar arrangement of hydroxyl and amino groups is present in the pyrimidine ring (21).

The action of phosphorus pentachloride and phosphorus oxychloride on leucopterin produces a monochloro derivative (XX) which can be hydrolyzed to leucopterin by alkalies (105). The chlorine of this leucopteryl chloride occupies the 6-position, since it is reduced by hydriodic acid to 6-desoxyleucopterin (XXI), which has been synthesized by the condensation of 2,4,5-triaminopyrimidine with oxalic acid (110).

[4] In accordance with the arbitrary use of aromatized formulas see page (64) this substance will be referred to in this review as "desaminoleucopterin."



Under the conditions which produce leucopteryl chloride from leucopterin, desaminoleucopterin yields a dichlorodihydroxypteridine, probably 2,6-dichloro-8,9-dihydroxypteridine,[5] but if alkali is avoided in working up the product, the corresponding tetrachloro compound results. This tetrachloropteridine reverts to the dichloro compound on treatment with 0.75 *N* sodium hydroxide at 80°C., and to desaminoleucopterin with 25 per cent sodium hydroxide at 140°C. With dry ammonia in ether, a monoaminotrichloropteridine is formed (87). The rather remarkable lability of the chlorine atoms in the 8- and 9-positions suggests that methyl groups in similar positions will be found to exhibit the characteristic reactivity in condensation reactions that is shown by methyl groups in other aromatic systems whose corresponding halogen derivatives are similarly active (2,4-dinitrotoluene and 2,4-dinitrochlorobenzene; α-picoline, α-chloropyridine; etc.). These positions, in the unsubstituted nucleus, may by similar reasoning be expected to be subject to fairly easy nucleophilic substitution.

Leucopterin does not react with diazomethane in dry ether (102, 105) but if aqueous methanol is added, nitrogen is evolved and two isomeric trimethyl derivatives are produced, one of which was of considerable importance in structural studies of the pterins, since it is soluble enough to allow molecular-weight determinations in phenol (102). Desaminoleucopterin yields a tetramethyl derivative under the same conditions (102).

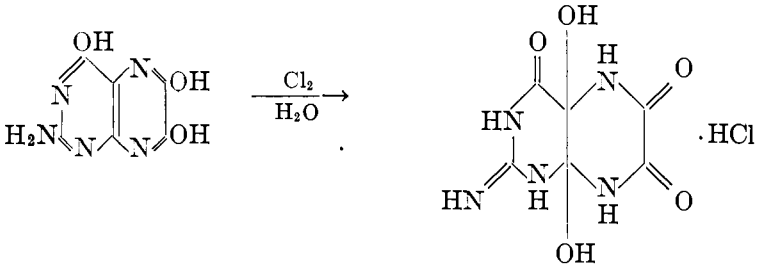
Pure leucopterin does not reduce phosphotungstic acid nor alkaline silver solutions (104), although earlier reports (88, 105), based on experiments with natural leucopterin probably containing uric acid, indicated that weak reduction of both occurred. It is attacked by alkaline ferricyanide (105), but in contrast to uric

[5] This structure was favored by Schöpf (87), primarily because the chlorine atoms in the analogous 4,5-diamino-2,6-dichloropyrimidine are stable to hydrolysis. The character of such chlorine atoms may be entirely altered, however, by the conversion of two primary amino groups to two tertiary ring members, and the structure of this substance cannot be considered firmly established.

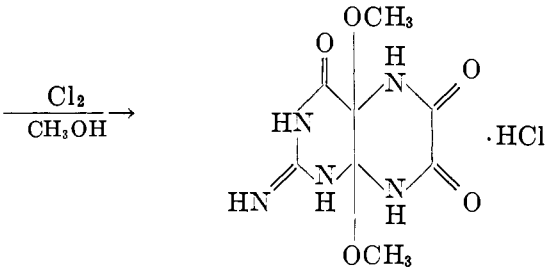
acid is unaffected by molecular oxygen in the presence of manganese dioxide (105).

Like uric acid, leucopterin is attacked at the juncture of the two rings by chlorine in methanol or by chlorine water. In methanol, the hydrochloride (XXIII) of leucopterin glycol dimethyl ether results, although this substance has not been obtained pure because of its ready loss of one methyl group by hydrolysis. The corresponding free base can be obtained from the hydrochloride by the action of pyridine in methanol (105). In water, the hydrochloride (XXII) of leucopterin glycol itself is obtained, along with a small amount of oxalylguanidine, the yield of which can be increased by working at 60–70°C. (102, 103, 105). Leucopterin glycol can be obtained from the hydrochloride by treatment with pyridine or by precipitating it as its double salt with mercuric acetate, followed by removal of mercuric ion by hydrogen sulfide (105).

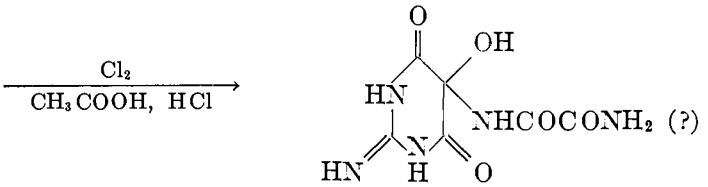
Leucopterin in suspension in acetic and hydrochloric acids on treatment with chlorine yields a product whose constitution has not definitely been established, but which may be 2-imino-5-hydroxyuramil-7-oxamide (XXIV), analogous to 5-hydroxypseudouric acid (107). Similarly, desaminoleucopterin, on treatment



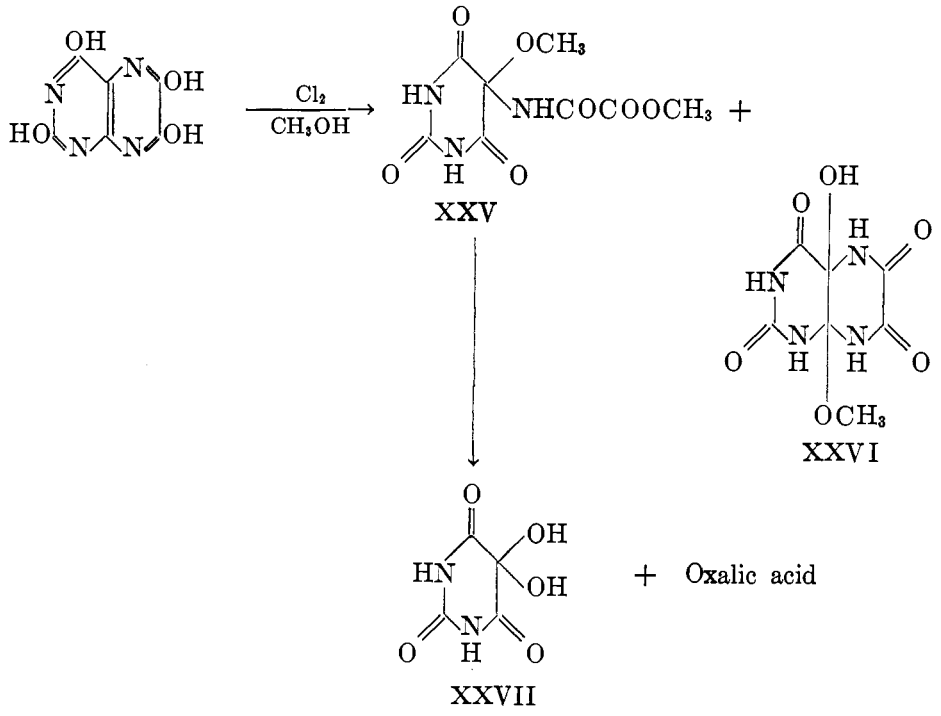
XXII



XXIII



XXIV



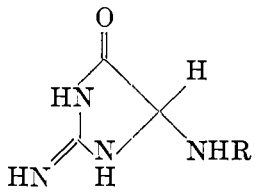
with chlorine in methanol, gives principally 5-methoxyuramil-7-oxalic acid methyl ester (XXV), although a small amount of the glycol half-ether (XXVI) corresponding to leucopterin glycol half-ether is also produced (109). On hydrolysis, the ester gives oxalic acid and alloxan (XXVII) (109).

Both leucopterin glycol and its diether are much less stable toward hydrolysis than leucopterin itself. The diether on warming in aqueous solution readily loses methanol, and further hydrolysis produces carbon dioxide and a small amount of guanidine, but no oxalic acid or ammonia. It is possible to isolate from the hydrolytic mixture by means of its double salt with mercuric chloride an acid $C_4H_5O_4N_2(OCH_3)$ which forms a crystalline methyl ester with diazomethane (105). Its constitution is not known with certainty, but it yields glyoxylic acid on alkaline hydrolysis and oxalic acid on acid hydrolysis and may have the accompanying structure (105):

$$NH_2COCH(OCH_3)NHCOCOOH$$

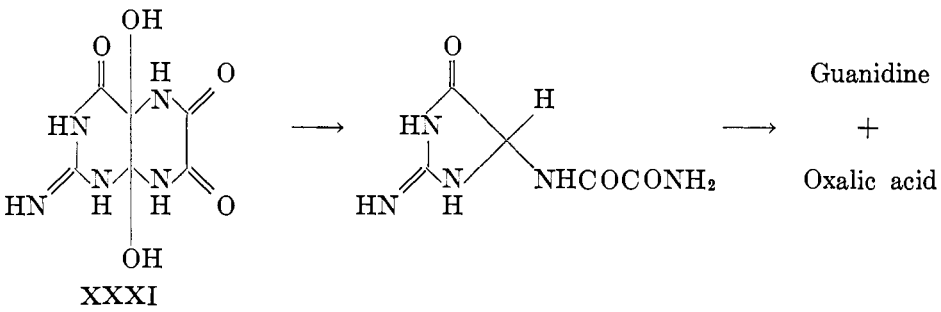
Leucopterin glycol is stable to boiling water (103) but on energetic hydrolysis with dilute acids it yields carbon dioxide, acid ammonium oxalate, and guanidine, isolated as its chloroaurate (105). Apparently the pyrimidine ring is in part altered before the liberation of guanidine, since only about one third of a mole of guanidine, which is stable under these conditions, is isolable (105). With excess

alkali, the glycol is degraded in the cold, carbon dioxide is split off, and it is possible to isolate 2-iminohydantoin-5-oxamide (XXVIII), the corresponding oxamic acid (XXIX), 2-imino-5-aminohydantoin (XXX) (103, 107), and oxalic acid (105).

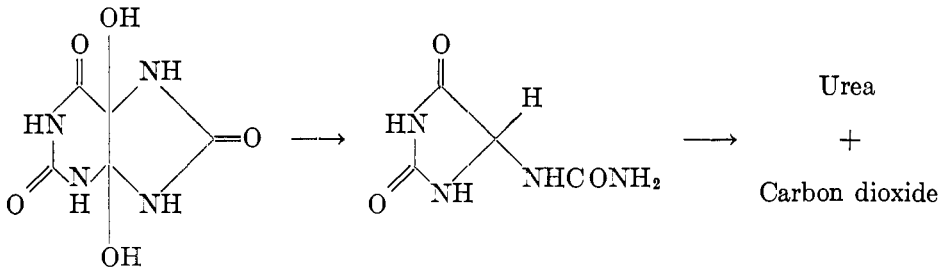


XXVIII : R=COCONH$_2$
XXIX : R=COCOOH
XXX : R=H

The degradation of leucopterin glycol (XXXI) is thus entirely analogous to that of uric acid glycol (XXXII) (81).



XXXI



XXXII

Guanidine can likewise be detected after treatment of 2-amino-6-hydroxy-8-pteridinecarboxylic acid with chlorine water and hydrolysis (2).

Leucopterin itself is completely degraded to carbon monoxide, carbon dioxide, ammonia, and glycine by vigorous acid hydrolysis (105).

The ordinary reducing agents do not attack leucopterin (105), although it has been possible to convert it in good yield into a mixture of xanthopterin and

dihydroxanthopterin by making use of sodium amalgam (92), and this reaction is employed in what appears to be the most convenient synthesis of xanthopterin. Electrolytic reduction of leucopterin produces a small amount of isoxanthopterin but no xanthopterin (104).

V. The Pterins[6]

A. General remarks on the pterins

As early as 1871 Meldola (quoted in reference 31) called attention to the fact that the yellow pigment in the wings of the common English brimstone butterfly (*Gonopterex rhamni*) was soluble in water. During the years 1889–93 F. Gowland Hopkins undertook a study of the wing pigments of the Pierids (29–32). He was able to isolate the white wing pigment of European cabbage butterflies (*Pieris brassicae*) in crystalline form, but mistook it for uric acid, to which it shows many superficial similarities. He also studied the yellow pigment from the wings of brimstone butterflies and showed that a similar red pigment occurs in certain other butterflies. The white, yellow, and red pigments all occur in the wing scales, and in this respect differ from a green pigment occurring between the chitin layers, later shown to be a chromoprotein whose blue prosthetic group is related to the bile pigments.

After a lapse of over thirty years Wieland and his collaborators undertook the study of these pigments. They were successful in isolating the yellow pigment of brimstone butterflies, which they named "xanthopterin," and its barium salt in pure crystalline state (108); shortly thereafter they demonstrated that the white pigment of cabbage butterflies which they called "leucopterin," while indeed similar to uric acid, differed from it in a number of ways (88). Continued work with these and other pierids by both Wieland and by Schöpf and their collaborators led to the isolation of several other pigments, of which isoxanthopterin (desoxyleucopterin) (105), formerly called "anhydroleucopterin," and erythropterin (83), the red pigment also observed by Hopkins, are the best characterized. The former accompanies leucopterin in small amounts in the wings of cabbage butterflies, and the latter accompanies xanthopterin in varying amounts in orange and red pierids. Several purine derivatives were also observed, among them uric acid (91), xanthine (72), hypoxanthine (91), and isoguanine, which was at first thought to be a pterin and was called "guanopterin" (83).

Of the pterins, xanthopterin is by far the most widespread in nature. Its color, fluorescence, and adsorption characteristics render it particularly easy to identify in small amounts (3). It occurs in insects of several other orders beside the lepidoptera, although only in a few species. Koschara (40) has shown that in small amounts it is a normal constituent of urine, both animal and human, and that it occurs in other animal sources but apparently not in plants (42). It has also been detected in the crab *Cancer pagurus* (69).

The occurrence of leucopterin appears to be limited to insects. In addition

[6] An excellent account of chemical work in the pterin field has been given by Schöpf (81).

to being present in both white and colored pierids (32, 78, 83, 88), it occurs in wasps (83) and is thus, like xanthopterin, not limited to the lepidoptera. It does not occur in human urine, guano, or snake excrement (17, 104).

It is remarkable that whereas the pterins appear to be end-products of protein metabolism laid down by the chrysalis during metamorphosis (104), no pterins appear in the excreta of adult insects which carry them as pigments (3, 105), although much uric acid is found.

Several other substances which are less well characterized than the simple pterins but which appear to be related to them have been isolated from widely differing sources. Koschara has obtained from human urine a sulfur-containing substance, $C_{11}H_{13}O_3N_5S_2$, which he has named urothione and whose absorption spectrum indicates the presence of a pteridine nucleus, although it gives no murexide test. It is optically active, contains a terminal glycol group revealed by periodic acid oxidation, and yields a tetraacetate in which one acetyl group is attached to nitrogen (43, 44). Polonovski and his coworkers have isolated by acid extraction of carp scales a substance, fluorescyanine, which may be related to the pterins. It contains less nitrogen, appears to be more soluble in organic solvents, and may be separated from riboflavin, with which it usually occurs, by chromatographic adsorption (66). Finally, fluorescent substances whose chemical and spectroscopic properties are similar to those of xanthopterin have been isolated from fluorescent bacteria (25), from the eyes of dogfish and alligators (62), and from the hepatopancreas of crabs (69).

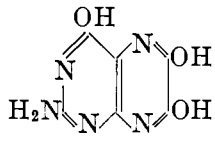
B. STRUCTURAL DETERMINATION AND SYNTHESES

Much of the chemical work leading to the elucidation of the structure of the pterins has already been discussed in the preceding section, and it will be necessary here only to give a brief account of how these observations were used in the structural studies. The preceding section, however, gave no inkling of the difficulties[7] encountered by Wieland, Schöpf, and their coworkers during the course of this work. The pigments are all quite sparingly soluble in organic solvents and are difficult to purify. The ordinary criteria of purity and identity are lacking, since the pterins are all very high melting. In addition to retaining water of crystallization tenaciously, they burn with difficulty in both carbon–hydrogen and Dumas nitrogen analyses. To point out that in spite of continuing work since 1925, the correct molecular formulas of the three most important pterins

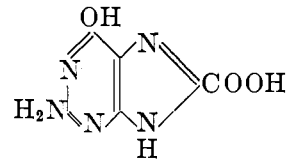
[7] No inconsiderable part was the collection of enough insects to insure reasonable quantities of material with which to work. A large number of cabbage butterflies were collected by school children during the summers of 1930–32 under the supervision of their teachers at the inducement of the Bavarian Ministry of Education, and a considerable number were also obtained from the Freiburg region. Around 200,000 insects were thus made available to Wieland and his collaborators at an early stage of the work (105). Schöpf and Kotler (85) used 16,000 of the large yellow South American Pierid, *Catopsila rurina*, in their study of xanthopterin, and Tartter (91) has reported results obtained during a study in which 850,000 insects (mixed Pierids) were used. Hopkins' work in this field was constantly hampered by scarcity of material (33).

were not known with certainty until 1941 (102), when the first reliable molecular-weight determination was carried out on a derivative of leucopterin, is merely to emphasize the difficulties encountered in the characterization of these substances.

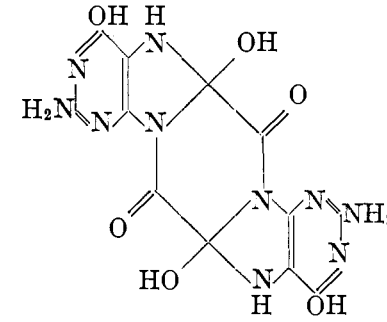
Early hypotheses (88, 108) as to the structures of leucopterin and xanthopterin, which postulated a basic structure made up of two 2,6-dioxypurine rings joined through the 8-positions, corresponding to $C_{10}H_{10}O_6N_8$ and $C_{10}H_{10}O_4N_8$ for these substances, were discarded when it was shown that guanidine could be obtained by chlorine–methanol oxidation of leucopterin, followed by hydrolysis of the resulting glycol ether (105). The analyses then available (82, 105) corresponded most closely to the formulas $C_{19}H_{19}O_{11}N_{15}$ for leucopterin and $C_{19}H_{19}O_7N_{15}$ for xanthopterin, although in 1936 that of xanthopterin was revised, on the basis of further analyses of its barium salt, to $C_{19}H_{18}O_6N_{16}$. Only after it had been demonstrated (106, 107) that xanthopterin could be oxidized to leucopterin by hydrogen peroxide or by molecular oxygen over platinum[8] was it apparent that the earlier analytical data were faulty. More rigorous drying (170°C. in vacuum) of leucopterin was then found to yield material whose analyses agreed with the formula $C_{18}H_{15}O_9N_{15}$ (or $C_6H_5O_3N_5$), forcing a revision of the empirical formula of xanthopterin to $C_{18}H_{15}O_6N_{15}$ (or $C_6H_5O_2N_5$) (107). At the same time, it was recognized that certain degradation products of leucopterin, obtained by careful alkaline hydrolysis of leucopterin glycol, which at an earlier time were thought to have contained 13, 14, and 15 carbon atoms (103), were in fact much simpler, and contained no more than five carbon atoms. Continued consideration of the trimeric formulas was therefore unnecessary (107). The demonstration by Purrmann (73) in 1940 that leucopterin can be synthesized in high yield from 2,4,5-triamino-6-hydroxypyrimidine and oxalic acid then limited the possible structures for this substance to XXXIII, XXXIV or, less probably, the dimeric modification of the latter, XXXV.



XXXIII



XXXIV

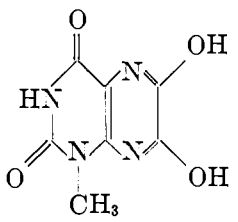


XXXV

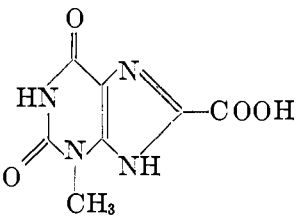
A choice between the first two of the above structures was made possible by Purrmann's observation (74) that the substance XXXVI, prepared from 4,5-

[8] As early as 1933 Schöpf had suggested that leucopterin might be an oxidation product of xanthopterin (82).

diamino-2,6-dihydroxy-3-methylpyrimidine and oxalic acid by his procedure, is not identical with Traube's (97) 3-methyl-8-xanthinecarboxylic acid



XXXVI



XXXVII

(XXXVII), which is easily decarboxylated in contrast to Purrmann's compound and to leucopterin both of which are very stable to heat. Further evidence in favor of the pyrimidinopyrazine structure was obtained by Schöpf (87), who converted desaminoleucopterin to a tetrachloropteridine by the action of phosphorus pentachloride, a reaction difficult to interpret on the basis of the 8-carboxypurine structure.

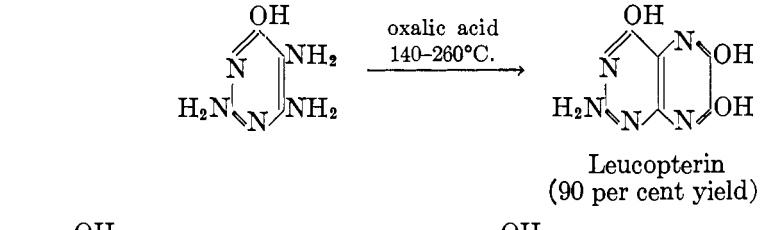
The dimeric formulation was finally definitely excluded by the preparation (102) of two isomeric trimethyl derivatives of leucopterin by the action of diazomethane in the presence of aqueous methanol, one of which in phenol provided the first reliable molecular-weight determination in an indifferent solvent in the entire pterin field.

Syntheses of xanthopterin and isoxanthopterin (8-desoxyleucopterin) by Purrmann (74, 75) completed the structural elucidation of the three well-characterized simple pterins. The condensation of 2,4,5-triamino-6-hydroxypyrimidine with dichloroacetic acid at 120°C. yielded the intermediate 5-dichloroacetylamino-2,4-diamino-6-hydroxypyrimidine, whose silver salt when heated with silver carbonate cyclized to xanthopterin. The over-all yield was only 6 per cent, principally because of the poor conversion in the last step (74). The structure of the intermediate acylated pyrimidine, on which the structure of xanthopterin rested,[9] was assigned on the basis of the known reactivity of the amino group in the 5-position. Isoxanthopterin was synthesized (75) by the condensation of 2,4,5-triamino-6-hydroxypyrimidine and mesoxalic ester in dilute acetic acid, followed by hydrolysis to give largely isoxanthopterincarboxylic acid and a small

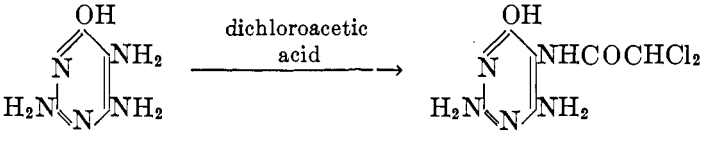
[9] It may be noted, however, that the structure of xanthopterin, and therefore of isoxanthopterin, no longer rests solely on the above assumption, since it has recently been shown (2) by means of the degradation of Weijlard, Tishler, and Erickson (100), that the methyl group in the aminohydroxymethylpteridine obtained as a degradation product of the *L. casei* fermentation factor (see page 91) is definitely at the 8-position. This methyl derivative has been oxidized to 2-amino-6-hydroxy-8-pteridinecarboxylic acid (2), which in turn has been synthesized (2) from Purrmann's isoxanthopterincarboxylic acid (75), the hydroxyl group of which must therefore occupy the 9-position. Its isomer, xanthopterincarboxylic acid, which can be converted through its dihydro compound to xanthopterin, thus carries its hydroxyl in the 8-position.

amount of xanthopterincarboxylic acid. The former decarboxylates readily on heating to 260°C. to give isoxanthopterin in excellent yield. If the condensation is carried out in 2 normal sulfuric acid, xanthopterincarboxylic acid is the principal product, possibly because the amino group in the 5-position of the pyrimidine ring is present as a salt in this medium (75). Xanthopterincarboxylic acid cannot be decarboxylated, but its easily prepared dihydro derivative is readily decarboxylated to yield dihydroxanthopterin, from which xanthopterin can be obtained by oxidation (75, 92).

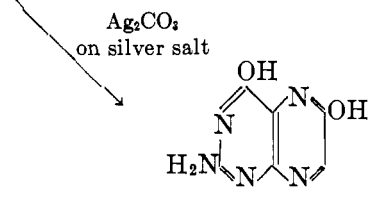
Xanthopterin has also been synthesized from 2,4,5-triamino-6-hydroxypyrimidine and glyoxylic acid–barium bisulfite by condensation in 78 per cent sulfuric acid (42), and by the reduction of synthetic leucopterin by means of an excess of sodium amalgam to give dihydroxanthopterin, which is in turn oxidized to xanthopterin by alkaline silver solutions (92). The use of 4,5-diamino-2,6-dihydroxypyrimidine and oxalic acid in Purrmann's synthesis leads to desaminoleucopterin in 62 per cent yield. All three of the well-characterized natural pterins—leucopterin, xanthopterin, and isoxanthopterin—can now be considered readily available. A summary of these synthetic methods follows:



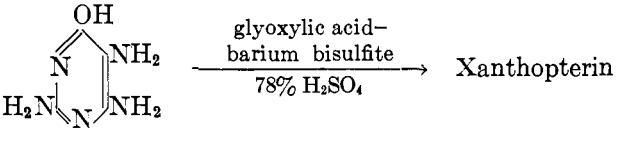
Leucopterin
(90 per cent yield)

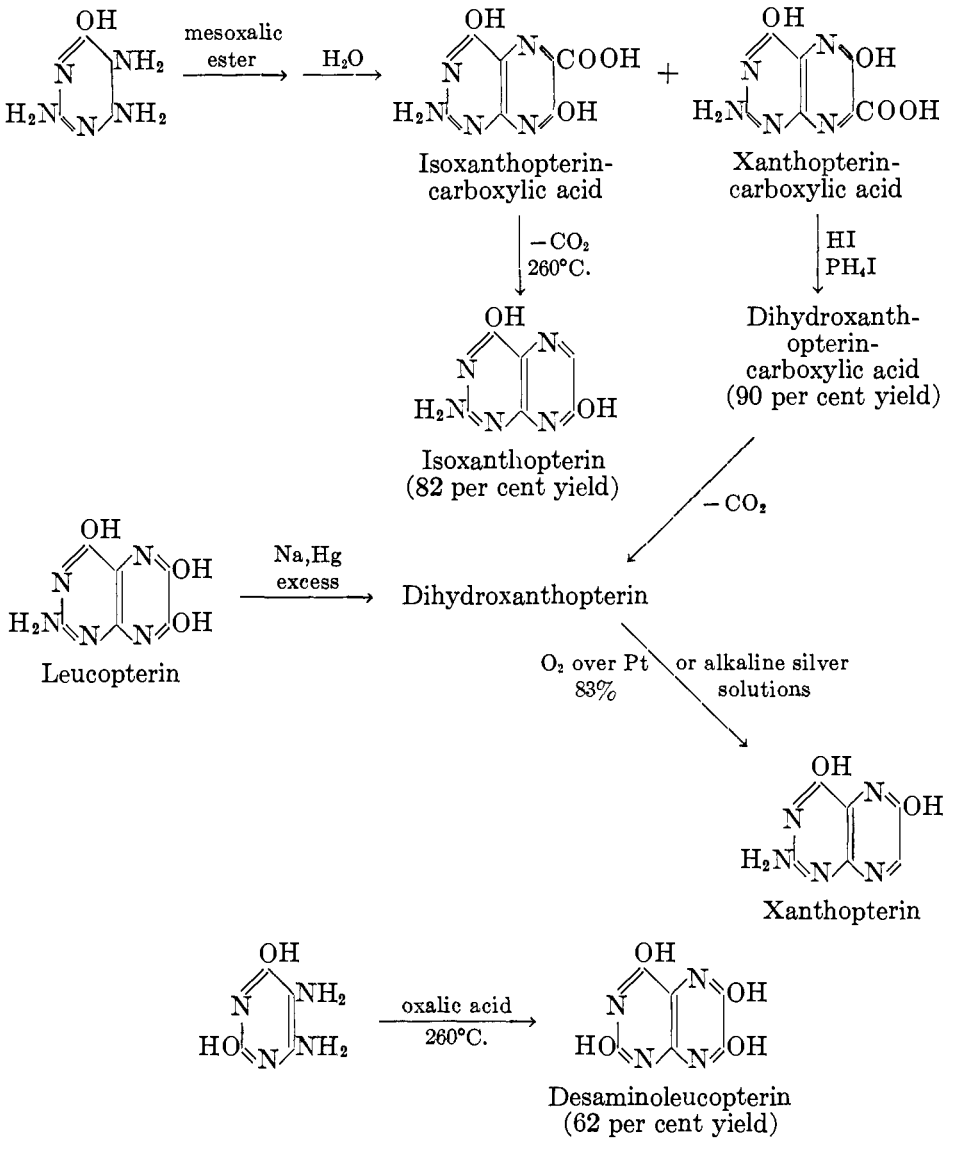


(60 per cent yield)



Xanthopterin
(10 per cent yield)





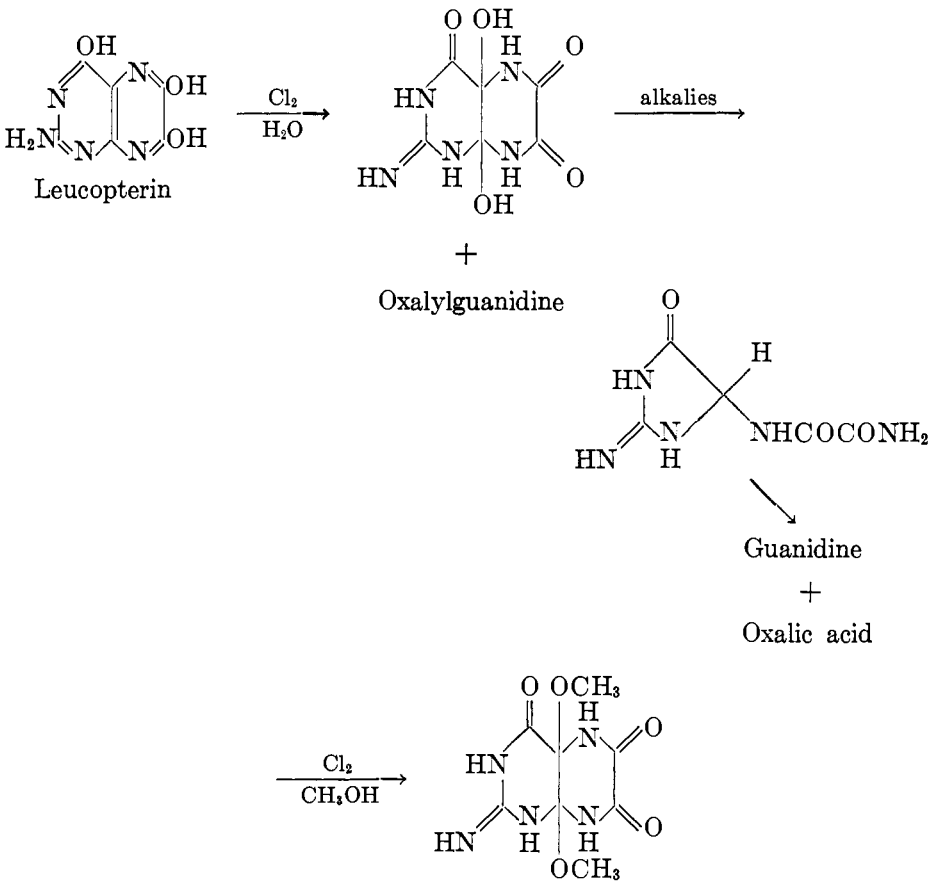
The degradative reactions leading to the recognition of the presence of a 2-aminopyrimidine ring in the pterins were for the most part carried out on leucopterin. With chlorine water it is smoothly oxidized at the ring fusion to yield leucopterin glycol, together with a small amount of oxalylguanidine, the yield of which can be increased by working at higher temperatures (102, 105). In methanol, an analogous reaction yields leucopterin glycol dimethyl ether (105). Similar oxidations of uric acid are well known. In contrast to the resistant leucopterin, which on energetic hydrolysis yields glycine, ammonia, carbon di-

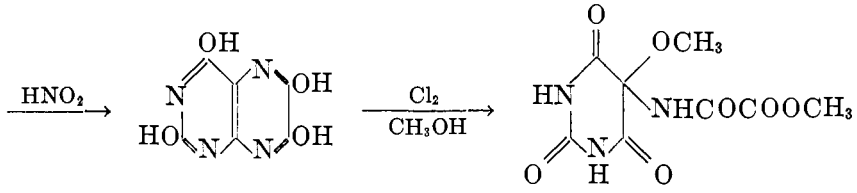
oxide, and carbon monoxide (105), the glycol and its ether are easily hydrolyzed to yield guanidine and oxalic acid. The corresponding uric acid glycol yields urea and carbon dioxide. Careful alkaline hydrolysis of leucopterin glycol yields 2-iminohydantoinoxamide, the corresponding oxamic acid, and 2-imino-5-aminohydantoin, at first mistaken for fragments with from 13 to 15 carbon atoms (103, 107). Similar treatment of uric acid glycol yields allantoin.

The action of chlorine in methanol on desaminoleucopterin (109) yields as a principal product 5-methoxyuramil-7-oxalic acid methyl ester, which contains all of the carbon atoms of the original substance and an intact pyrimidine ring.

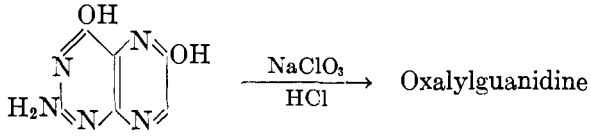
The presence of a 2-aminopyrimidine ring in xanthopterin may likewise be inferred from the fact that both it and 2,4,5-triamino-6-hydroxypyrimidine yield oxalylguanidine and oxalic acid by the action of sodium chlorate in acid solutions (85).

A résumé of these reactions (most of which are also illustrated on pages 75, 76, and 77) follows:





Desaminoleucopterin



Xanthopterin

The striking dissimilarity between xanthopterin and isoxanthopterin is manifested in a variety of ways.[10] Physically, xanthopterin is yellow and exhibits marked basic properties, whereas isoxanthopterin is colorless and only slightly basic. Great similarity in color and fluorescence exists between xanthopterin and the flavines, whereas isoxanthopterin and leucopterin show no such similarity. Unlike xanthopterin, isoxanthopterin cannot be oxidized to leucopterin by catalytically activated oxygen, but is converted to leucopterin glycol by the action of chlorine water. Finally, the difference in behavior toward fuming hydriodic acid of the dihydro compounds (see page 72) and the much greater ease of decarboxylation of isoxanthopterincarboxylic acid may be mentioned.

Erythropterin, the red pigment which accompanies xanthopterin in orange and red pierids, is much less well characterized than the foregoing compounds. The free pterin is not readily crystallizable, no crystalline salts with either acids or bases have been obtained (83), and its empirical formula is not yet known with certainty. It gives no murexide reaction, is less basic than xanthopterin, and can be separated from xanthopterin by crystallization from dilute hydrochloric acid (83).

The chromophoric system of erythropterin is highly stable to hydrosulfite (83), but reduction to a colorless leuco compound results with zinc dust and formic acid (84). Energetic acid hydrolysis yields 11–14 per cent of glycine, although its leuco compound yields 53 per cent under the same conditions (84). Its spectrum resembles that of murexide (83).

Erythropterin preparations have a tendency to give deeply colored transformation products of low solubility in ammonia (83) but there is evidence that this is not exclusively a property of erythropterin (78) and whether or not the change involves erythropterin or an impurity is not yet certain.

[10] These differences are discussed more fully on page 72.

C. PTERORHODIN

The production of a red-violet transformation product as a result of the action of acids on crude pterins was first noted by Hopkins (32).[11] Later workers also noticed a deeply colored by-product (40, 82, 83, 107). Its formation involves oxidation of some component of crude pterins by molecular oxygen (33, 107) or by hydrogen peroxide or chlorine (78).

The substance is insoluble in water and dilute acids and in all ordinary organic solvents. It is soluble in concentrated sulfuric acid to give a brick-red solution with a violet-red tinge, and on dilution of this solution a crystalline sulfate can be isolated. Further dilution results in complete hydrolysis. It is soluble in, but slowly decomposed by, dilute alkalies (78).

The work of Schöpf (82, 83) would indicate that erythropterin, whose constitution is not yet known, may be a precursor of pterorhodin, but crystalline xanthopterin barium salt (from *Catopsilia argante*) containing no erythropterin likewise can be made to yield the pigment (107), whereas synthetic xanthopterin cannot (78). It seems clear, as Purrmann points out (78), that xanthopterin is not a precursor of pterorhodin and that a stubborn impurity, not erythropterin, in natural xanthopterin from some sources may be. Whether erythropterin is also a procursor remains undecided.

No investigation of this red-violet pigment was made until quite recently, when Hopkins (33), the original discoverer, returned to its study after a lapse of forty-seven years. He was able to outline with more precision the conditions under which pterorhodin is formed, and showed that air oxidation at pH values of 4.6 and lower of both crude erythropterin and of xanthopterin (from natural sources) purified through its barium salt, resulted in production of pterorhodin. Crude natural xanthopterin yields pterorhodin with much less ease than material purified through the barium salt, and Hopkins suggested that "native" xanthopterin probably does not yield pterorhodin, but is altered during purification. Purrmann's results (78) indicate, however, that purification of xanthopterin through the barium salt probably concentrates the tenacious impurity responsible for pterorhodin formation. Perhydrol oxidation of pterorhodin in dilute ammonia gives colorless water-soluble products, but Hopkins was unable to identify these because of lack of material.

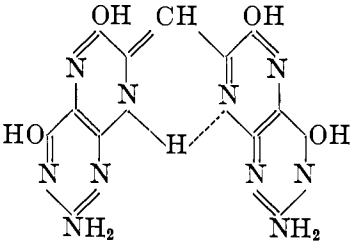
Purrmann was able largely to clear up the question of the structure of pterorhodin in a brilliant paper published in 1944 (78). Pterorhodin is stable to alkaline hydrosulfite and to fuming hydriodic acid, but a colorless leuco compound can be prepared from it by the action of sodium amalgam. This leuco compound forms nicely crystalline salts with acids, and analyses of the perchlorate, sulfate, chloride, and iodide all agree with the formula $C_{13}H_{14}O_4N_{10}$ for the leuco compound and thus indicate $C_{13}H_{10}O_4N_{10}$ for pterorhodin, with which its analyses and those of its sulfate also agree. Pterorhodin gives oxalylguanidine in 56 per cent of the

[11] Hopkins, who suggested the no longer suitable name "lepidoporphyrin" in 1895 for this substance, has recently proposed the name "rhodopterin"; but Purrmann (78), after elucidation of its structure, believes "pterorhodin" is preferable, in as much as the material is not a simple pterin.

amount required for two 2-aminopyrimidine rings under conditions which yield 60 per cent from leucopterin.

Oxidation of pterorhodin by hydrogen peroxide in concentrated sulfuric acid gave two products, leucopterin and xanthopterincarboxylic acid, which between them account for all the carbon and nitrogen atoms of pterorhodin. They were isolated by making use of the insolubility of the potassium salt of leucopterin in normal potassium carbonate, in which xanthopterincarboxylic acid is soluble.

On the basis of these observations, Purrmann proposed the accompanying



XXXVIII

Pterorhodin

structure for pterorhodin (XXXVIII), although he does not consider the structure established beyond question, since the leucopterin and xanthopterincarboxylic acid isolated did not amount to half of the pterorhodin taken, and xanthopterincarboxylic acid is converted to leucopterin, although only slowly, under the conditions of the degradation.

Unlike the other pterins of known structure, the precursor of pterorhodin appears to contain more than six carbon atoms. It is similar to the known pterins in the configuration of the pyrimidine ring, is a somewhat stronger acid than xanthopterin, and is not identical with leucopterorhodin (78).

D. PHYSIOLOGICAL ASPECTS OF THE PTERINS

During the time the structural work on the pterins was being carried out, accounts pointing toward a definite physiological importance of members of this class, particularly xanthopterin, began to appear. Koschara's demonstration (40) of the occurrence of xanthopterin in human urine undoubtedly stimulated work in this direction. A suggestion that xanthopterin was in some way related to nutritional anemia was received as early as 1936, when Tschesche and Wolf (98, 99) observed that pterins, particularly xanthopterin, possessed hematopoietic activity in young rats fed an exclusive diet of goat's milk. On this diet, the animals develop an anemia reminiscent of sprue characterized by a blood picture resembling that of pernicious anemia (98). About the same time, Italian workers (55), during a chemical investigation of the antipernicious anemia factor of liver, obtained two fractions after a lengthy process of fractionation which, when combined, had appreciable action against pernicious anemia. One of these fractions was thought to contain a pterin, the other a nucleotide and a polypeptide.

In 1939 Koschara observed an increase in urinary secretion of xanthopterin

during a variety of disorders of the hematopoietic system (as well as in some other pathological conditions) (46). The distribution of xanthopterin in many tissues in both well and diseased men has been studied by this author (45, 46) who, because of the parallelism in occurrence between riboflavin and xanthopterin, has suggested that these two pigments may act as a functional unit (45).

A further indication of the relationship between the pterins and hematopoiesis appears in the work of Jacobson, who has shown not only that xanthopterin and leucopterin evoke a reticulocyte response in splenectomized rabbits (38), but also that the argentaffine cells of stomach and intestinal epithelium, whose location agrees with that of the substance active against pernicious anemia, contain a pterin (37) the intensity of whose fluorescence spectrum parallels hematopoietic activity (39).

Xanthopterin is also hematopoietically active in anemic fingerling salmon (59, 89).

Two independent observations indicate that yeast, whose content of preformed folic acid is low, and liver, grass, and urine, when incubated with fresh liver, produce folic acid (94, 114) and that these materials therefore contain a precursor of folic acid. The stability to heat and the adsorption–elution characteristics of this precursor are similar to those of xanthopterin (114). Synthetic xanthopterin, but not leucopterin, shows the effect.

In spite of the association of xanthopterin with nutritional anemia which is evident from the foregoing, a great deal of evidence exists indicating that while xanthopterin may possess hematopoietic activity, it alone is not sufficient to bring about remission of these anemias in all species. Thus it has been shown that rapid regeneration of a normal blood picture in dogs rendered anemic by phlebotomy occurs when dry or whole liver is added to a whole milk diet, but that the liver can not be wholly replaced by, among other things, xanthopterin (57). Likewise, while xanthopterin delays the development of nutritional anemia in monkeys and produces a hematopoietic response in anemic animals, it does not offer full protection against vitamin M deficiency (see page 89) (93, 96) nor can it replace vitamin B_c in the diet of the chick (60). Furthermore, whereas xanthopterin appears to produce an immediate gain in weight and a pronounced leucocyte response in rats rendered leucopenic by succinylsulfathiazole (93; see also, however, 114), distribution of the white cells may not be normal after this therapy (93).

In addition to the association of xanthopterin with hematopoiesis, there are other indications that this class of compounds is of physiological importance. Polanovski and his coworkers have shown that a number of pteridine derivatives of rather divergent structures possess vitamin B_1 activity of a low order in rats and pigeons but not in the flagellate *Polytomella caeca*. Fluorescyanine, a pigment possibly related to the pterins, which has been isolated by these workers from carp scales has a similar but stronger action (8, 9, 10, 11, 12, 67).

Xanthopterin and especially folic acid also appear to possess a growth-inhibiting effect on malignant tumors in mice (26, 54).

The results of a pharmacological investigation of xanthopterin have been published by Hörlein (27).

VI. Folic Acid[12]

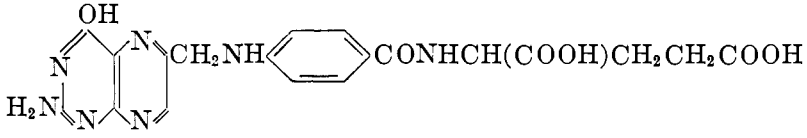
A. DIETARY FACTORS RELATED TO FOLIC ACID AND THEIR INTERRELATIONSHIPS

The discovery of folic acid, one of the most recently recognized members of the vitamin B complex, was the result of nutritional work, both bacterial and animal, in several laboratories. Widely divergent approaches were used and the various lines of experimentation converged only at a relatively late stage in the investigations. No detailed account of the physiological work, which is treated thoroughly in the review of Berry and Spies (5), can be given here. It will be sufficient to say that two well-studied dietary factors for animals, i.e., "vitamin M," without which monkeys develop a fatal blood disorder characterized by leucopenia and sometimes anemia (53), and "vitamin B_c," lack of which results in poor growth and macrocytic hyperchromic anemia in chicks (28), and several growth factors for the bacteria *Lactobacillus casei* and *Streptococcus lactis* R (for a complete bibliography see reference 5) variously known as "norite eluate factor," "folic acid," "*L. casei* factor," "SLR factor," etc., are considered to be, if not identical, very closely related, and the name "folic acid" appears now to be applied indiscriminately to various members of the group. Crystalline substances corresponding to four of the above have been isolated, i.e., vitamin B_c from both liver and yeast (6, 63), vitamin B_c conjugate from yeast (64), and two *L. casei factors*, one from fermentation products and one from liver (35, 90).

B. THE *L. casei* LIVER FACTOR

1. Identity and physiological aspects

The *L. casei* factor from liver has been shown to be identical with vitamin B_c (65), and its structure (XXXIX) has been established by degradation and synthesis (1, 2). The other two crystalline substances, the *L. casei* fermentation factor and vitamin B_c conjugate, are peptide conjugates of this simpler molecule, which has been called "pteroylglutamic acid," and yield on hydrolysis two and six additional molecules of *l*(+)-glutamic acid, respectively. Pteroylglutamic acid is now commercially available, and its therapeutic value in various clinical types of macrocytic anemia and sprue has been clearly established (5, 15). The clinical evidence for its hematopoietic activity has been reviewed by Berry and Spies (5). It also has been shown to be active in nutritional anemias of monkeys (112; see also 16), giving the typical vitamin M response, and corrects or prevents the blood disorders produced in rats by succinylsulfathiazole (20) or thiourea and thyroxine



XXXIX

[12] An excellent review covering the nutritional and physiological aspects of the folic acid problem and the therapeutic applications of the *Lactobacillus casei* liver factor has recently been published by Berry and Spies (5).

(14). Both pteroylglutamic acid and the *L. casei* fermentation factors are active in the chick, possibly equally so on a molar basis (34), and it appears likely that pteroylglutamic acid (XXXIX) or various peptide conjugates of it with *l*(+)-glutamic acid are involved in all of the physiological effects mentioned above.

The occurrence of *p*-aminobenzoic acid, long a recognized member of the vitamin B complex, in combined form in "folic acid" is of great biochemical interest. It has been shown that the synthesis of pteroylglutamic acid from *p*-aminobenzoic acid is the site of sulfonamide inhibition in the organism *S. fecales* R (51). *p*-Aminobenzoic acid may be utilized in other ways than in the formation of pteroylglutamic acid, however, since the latter substance has less than 0.001 per cent of the activity of *p*-aminobenzoic acid for a mutant strain of *Escherichia coli* (52).

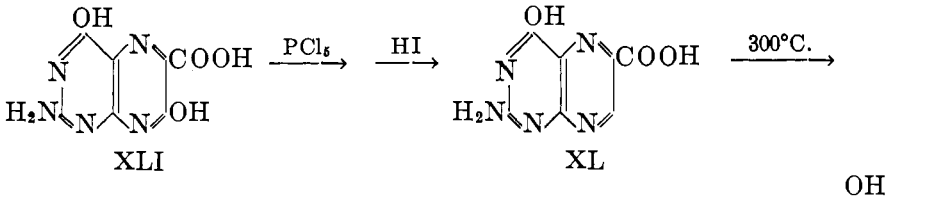
Folic acid concentrates have been reported to have tumor-inhibiting activity against malignant mouse tumors, chiefly mammary adenocarcinomas (26).

2. Degradation and synthesis

The elucidation of the structure[13] of the *L. casei* liver factor affords a splendid example of the application of information obtained in what might be regarded as a remote and possibly academic field, that of the pterins, to the solution of a problem of great importance for practical therapy. The timely completion of the structural work on the pterins in 1940 and 1941 provided the necessary knowledge for the rapid solution of the problem of structure and synthesis of the *L. casei* liver factor which must otherwise have been greatly delayed. A résumé of the degradative work follows (2):

The close relationship between the *L. casei* fermentation factor and the *L. casei* liver factor was established (2) by anaerobic alkaline hydrolysis by which the fermentation factor is converted into the *dl* liver factor (pteroylglutamic acid) with the simultaneous formation of 2 moles of glutamic acid. Similarly, enzymatic hydrolysis of vitamin B_c conjugate yields pteroylglutamic acid (64) and 6 moles of *l*(+)-glutamic acid are liberated by acid hydrolysis (65). Aerobic alkaline hydrolysis of the *L. casei* fermentation factor yields two fractions in equimolar amounts, one of which is a highly fluorescent dibasic acid (pK_a values 3.9 and 7.7) whose fluorescent behavior, ultraviolet-light absorption, and ultimate analysis coupled with the isolation of guanidine following oxidation with chlorine water and acid hydrolysis suggested for its structure a 2-aminopteridine nucleus carrying a hydroxyl and a carboxyl group. It was fully identified as 2-amino-6-hydroxy-8-pteridinecarboxylic acid (XL) by comparison with an authentic sample prepared from isoxanthopterincarboxylic acid (XLI) by chlorination with phosphorus pentachloride, followed by reduction with hydriodic acid. It can be decarboxylated at 300°C. to a monobasic acid, pK_a 8.0, which is identical with synthetic 2-amino-6-hydroxypteridine (XLII) prepared from 2,4,5-triamino-6-hydroxypyrimidine and glyoxal. The hydroxyl in the 9-position of isoxanthopterincarboxylic acid is thus the one removed in the chlorination and reduction described above.

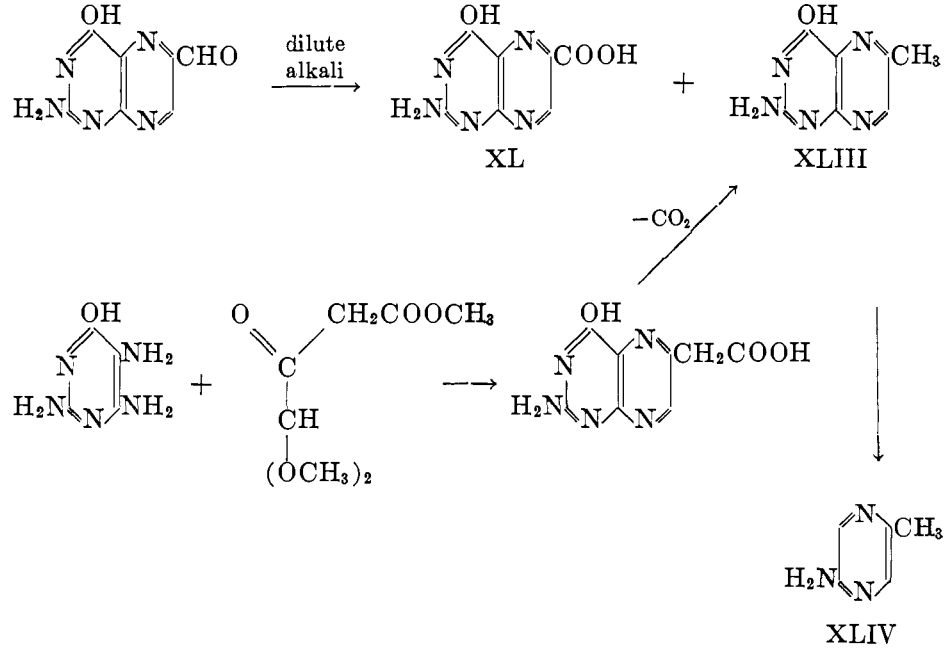
[13] Although the synthesis of the material was announced in August, 1945 (1), neither the structure nor the details of degradation and synthesis were disclosed for nearly a year (2).



XLI XL XLII

The second fraction from aerobic alkaline hydrolysis showed the presence of an aromatic amino group and yielded *p*-aminobenzoic acid after acid hydrolysis.

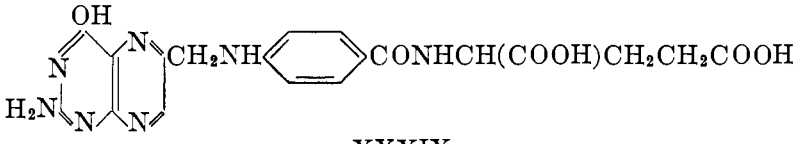
A second cleavage of the *L. casei* fermentation factor was achieved with the aid of sulfurous acid, and again yielded a pteridine fraction and an aromatic amine fraction. In this case, however, the pteridine fraction reacted readily with aldehyde reagents and on standing in dilute alkali in the absence of air underwent a dismutation to yield the carboxylic acid described above and 2-amino-6-hydroxy-8-methylpteridine (XLIII), identified by comparison with a sample synthesized by the condensation of 2,4,5-triamino-6-hydroxypyrimidine and methyl γ,γ-dimethoxyacetoacetate, followed by decarboxylation of the 2-amino-6-hydroxy-8-pteridineacetic acid so obtained. The methyl compound and the corresponding acetic acid can both be oxidized to the previously described 2-amino-6-hydroxy-8-pteridinecarboxylic acid (XL) by hot alkaline



permanganate. Degradation of the methyl compound by the method[14] of Weijlard, Tishler, and Erickson (100) gave 2-amino-5-methylpyrazine (XLIV), confirming the position of the methyl group.

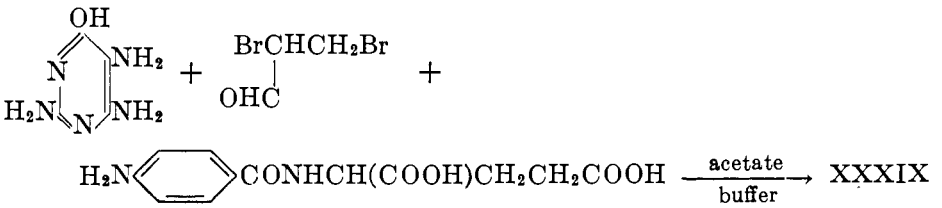
Acid hydrolysis of the aromatic amine fraction liberated three-fourths of the nitrogen as α-amino acid nitrogen, and again *p*-aminobenzoic acid was isolated. The presence of 3 moles of glutamic acid in the hydrolysate was indicated by microbiological assay. Aqueous hydrolysates of the *L. casei* fermentation factor had yielded pyrrolidonecarboxylic acid, a further indication of the presence of glutamic acid.

The necessity for oxygen in the alkaline cleavage, and the simultaneous liberation of an aromatic amino group and the pteridinecarboxylic acid in this reaction, suggests the linkage of the pteridine nucleus to the amino group through a methylene group; accordingly structure XXXIX was proposed for the *L. casei* liver factor.



XXXIX

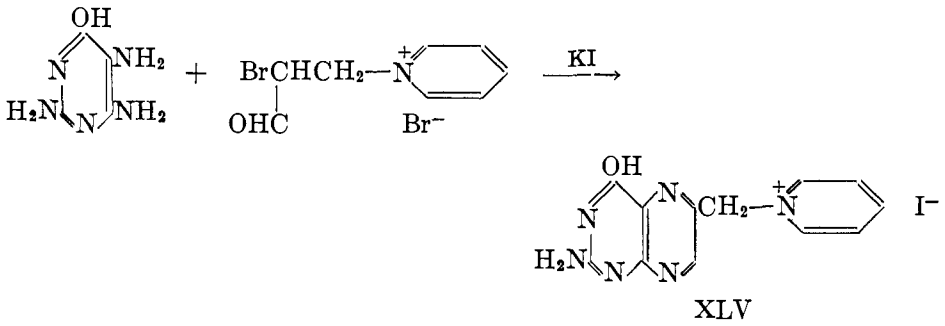
This structure was confirmed by two syntheses, both of which gave material identical in physical and physiological properties with the natural material (1, 2). The first synthesis was achieved by condensing equivalent amounts of 2,4,5-triamino-6-hydroxypyrimidine, α,β-dibromopropionaldehyde, and *p*-aminobenzoyl-*l*(+)-glutamic acid in an acetate buffer. The condensation proceeds by way of an intermediate dihydro compound which is oxidized during the course of the reaction, and yields 15 per cent of active material as determined by microbiological assay. A lengthy purification process affords the pure crystalline *L. casei* liver factor in unspecified yield.



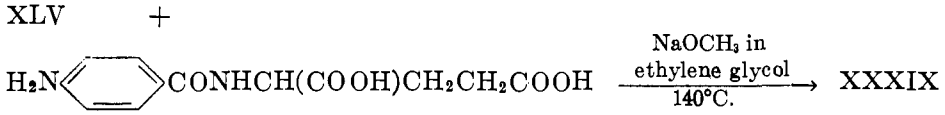
A second synthesis utilized the interaction in the presence of potassium iodide of 2,4,5-triamino-6-hydroxypyrimidine with the condensation product of pyridine and α,β-dibromopropionaldehyde to give *N*-[(2-amino-6-hydroxy-8-pteridyl)-methyl]pyridinium iodide (XLV), which was then condensed with *p*-aminobenzoyl-*l*(+)-glutamic acid by means of sodium methoxide in ethylene glycol at 140°C. The crude product again contained about 15 per cent of biologically

[14] Angier *et al.* (2) do not state which of the two methods described by these authors was used in the degradation.

active material, from which the crystalline product could be isolated by the same purification process.



XLV



That the pyridinium methyl group in the preliminary condensation product occupies the 8-position was shown by alkaline permanganate oxidation to 2-amino-6-hydroxy-8-pteridinecarboxylic acid (XL).

The same synthetic methods were used to prepare *N*-[(2-amino-6-hydroxy-8-pteridyl)methyl]-*p*-aminobenzoic acid ("pteroic acid"), which proved active for *S. fecalis* R but inactive for *L. casei* and for the chick (2).

Note added in proof: A publication describing in considerable detail the characterization of vitamin B_c and its isolation as the free acid and as its methyl ester from hog liver, horse liver, and from yeast has recently appeared (64a). Publications describing its degradation are in press.

VII. References

(1) Angier, R. B., *et al.*: Science **102,** 227 (1945).
(2) Angier, R. B., *et al.*: Science **103,** 667 (1946).
(3) Becker, E.: Z. physiol. Chem. **246,** 177 (1937).
(4) Becker, E., and Schöpf, C.: Ann. **524,** 124 (1936).
(5) Berry, L. J., and Spies, T. D.: Blood **1,** 271 (1946).
(6) Binckley, S. B., *et al.*: Science **100,** 36 (1944).
(7) Bloom, E. S., *et al.*: Science **100,** 295 (1944).
(8) Busnel, R., *et al.*: Compt. rend. **217,** 185 (1943).
(9) Busnel, R., *et al.*: Compt. rend. soc. biol. **137,** 594 (1943).
(10) Busnel, R., *et al.*: Compt. rend. soc. biol. **138,** 171 (1944).
(11) Busnel, R., *et al.*: Compt. rend. soc. biol. **138,** 366 (1944).
(12) Busnel, R., *et al.*: Compt. rend. soc. biol. **139,** 139 (1945).
(13) Cain, C. K., Mallette, M. F., and Taylor, E. C., Jr.: J. Am. Chem. Soc. **68,** 1996 (1946).
(14) Daft, F. S., Kornberg, A., Ashburn, L. L., and Sebrell, W. H.: Proc. Soc. Exptl. Biol. Med. **61,** 154 (1946).
(15) Darby, W. J., Jones, E., and Johnson, H. C.: J. Am. Med. Assoc. **130,** 780 (1946).
(16) Day, P. L., Mims, V., and Totter, J. R.: J. Biol. Chem. **161,** 45 (1945).
(17) Decker, P.: Z. physiol. Chem. **274,** 223 (1942).

(18) ELLINGER, P., AND KOSCHARA, W.: Ber. **66**, 315 (1933).
(19) ELLINGSON, R. C., HENRY, R. L., AND MACDONALD, F. G.: J. Am. Chem. Soc. **67**, 1711 (1945).
(20) ENDICOTT, K. M., DAFT, F. S., AND OTT, M.: Arch. Path. **40**, 364 (1945).
(21) FISCHER, E.: Ber. **30**, 2245 (1897).
(22) FROMHERZ, H., AND KOTZSCHMAR, A.: Ann. **534**, 283 (1938).
(23) GABRIEL, S., AND SONN, A.: Ber. **40**, 4857 (1907).
(24) GANAPATI, K.: J. Indian Chem. Soc. **14**, 627 (1937).
(25) GIRAL, F.: Anales soc. españ. fís. quím. **34**, 667 (1936).
(26) HESSELBACH, M. L., AND BURK, D.: Record Chem. Progress (Hooker Sci. Lib.) **5**, 37 (1944).
(27) HÖRLEIN, H.: Arch. exptl. Path. Pharmakol **198**, 258 (1941).
(28) HOGAN, A. G., AND PARROTT, E. M.: J. Biol. Chem. **132**, 507 (1940).
(29) HOPKINS, F. G.: Nature **40**, 335 (1889).
(30) HOPKINS, F. G.: Nature **45**, 197 (1891).
(31) HOPKINS, F. G.: Nature **45**, 581 (1892).
(32) HOPKINS, F. G.: Phil. Trans. Roy. Soc. **B186**, 661 (1893).
(33) HOPKINS, F. G.: Proc. Roy. Soc. (London) **B130**, 359 (1942).
(34) HUTCHINGS, B. L., OLESON, J. J., AND STOLSTAD, E. L. R.: J. Biol. Chem. **163**, 447 (1946).
(35) HUTCHINGS, B. L., *et al.*: Science **99**, 371 (1944).
(36) ISAY, O.: Ber. **39**, 250 (1906).
(37) JACOBSON, W.: J. Path. Bact. **49**, 1 (1939).
(38) JACOBSON, W., AND WILLIAMS, S. M.: J. Path. Bact. **57**, 423 (1945).
(39) JACOBSON, I. W., AND SIMPSON, D. M.: Biochem. J. **40**, 3 (1946).
(39a) KARRER, P., SCHWYZER, R., ERDEN, B., AND SIEGWART, A.: Helv. Chim. Acta **30**, 1031 (1947).
(40) KOSCHARA, W.: Z. physiol. Chem. **240**, 127 (1936).
(41) KOSCHARA, W.: Z. physiol. Chem. **250**, 161 (1937).
(42) KOSCHARA, W.: Z. physiol. Chem. **277**, 159 (1943).
(43) KOSCHARA, W.: Z. physiol. Chem. **277**, 284 (1943).
(44) KOSCHARA, W.: Z. physiol. Chem. **279**, 44 (1943).
(45) KOSCHARA, W., AND HAUG, H.: Z. physiol. Chem. **259**, 97 (1939).
(46) KOSCHARA, W., AND HRUBESCH, A.: Z. physiol. Chem. **258**, 39 (1939).
(47) KÜHLING, O.: Ber. **27**, 2116 (1894).
(48) KÜHLING, O.: Ber. **28**, 1970 (1895).
(49) KUHN, R., AND COOK, A. H.: Ber. **70**, 761 (1937).
(50) KUHN, R., GYÖRGY, P., AND WAGNER-JAUREGG, T.: Ber. **66**, 317 (1933).
(51) LAMPEN, J. O., AND JONES, M. J.: J. Biol. Chem. **164**, 485 (1946).
(52) LAMPEN, J. O., ROEPKE, R. R., AND JONES, M. J.: J. Biol. Chem. **164**, 789 (1946).
(53) LANGSTON, W. C., *et al.*: J. Exptl. Med. **68**, 923 (1938).
(54) LEWISOHN, R., *et al.*: Proc. Soc. Exptl. Biol. Med. **56**, 144 (1944).
(55) MAZZA, F. P., AND PENATI, F.: Arch. sci. biol. **23**, 443 (1937).
(56) MAZZA, F. P., AND TAPPI, G.: Arch. sci. biol. **25**, 438 (1939).
(57) MCKIBBIN, J. M., SCHAEFER, A. E., ELVEHJEM, C. A., AND HART, E. B.: J. Biol. Chem. **145**, 107 (1942).
(58) MITCHELL, H. K.: Science **97**, 442 (1943).
(59) NORRIS, E. R., AND SIMMONS, R. W.: J. Biol. Chem. **158**, 449 (1945).
(60) O'DELL, B. L., AND HOGAN, A. G.: J. Biol. Chem. **149**, 323 (1943).
(61) PATTERSON, A. M., AND CAPELL, L. T.: *The Ring Index*. Reinhold Publishing Corporation, New York (1940).
(62) PIRIE, A., AND SIMPSON, D. M.: Biochem. J. **40**, 14 (1946).
(63) PFIFFNER, J. J., *et al.*: Science **97**, 404 (1943).
(64) PFIFFNER, J. J., *et al.*: Science **102**, 228 (1945).
(64a) PFIFFNER, J. J., BINKLEY, S. B., BLOOM, E. S., AND O'DELL, B. L.: J. Am. Chem. Soc. **69**, 1476 (1947).

(65) PFIFFNER, J. J., CALKINS, D. G., BLOOM, E. S., AND O'DELL, B. L.: J. Am. Chem. Soc. **68,** 1392 (1946).
(66) POLONOVSKI, M., BUSNEL, R., AND PESSON, M.: Compt. rend. **217,** 163 (1943).
(67) POLONOVSKI, M., *et al.:* Compt. rend. **218,** 609 (1944).
(68) POLONOVSKI, M., PESSON, M., AND VIEILLEFOSSE, R.: Compt. rend. **218,** 796 (1944).
(69) POLONOVSKI, M., AND FOURNIER, E.: Compt. rend. soc. biol. **138,** 357 (1944).
(70) POLONOVSKI, M., VIELLEFOSSE, R., AND PESSON, M.: Bull. soc. chim. **12,** 78 (1945).
(71) POLONOVSKI, M., GUIN, S., PESSON, M., AND VIELLEFOSSE, R.: Bull. soc. chim. **12,** 924 (1945).
(72) PURRMANN, R.: Z. physiol. Chem. **260,** 105 (1939).
(73) PURRMANN, R.: Ann. **544,** 182 (1940).
(74) PURRMANN, R.: Ann. **546,** 98 (1940).
(75) PURRMANN, R.: Ann. **548,** 284 (1941).
(76) PURRMANN, R.: German patent 721,930.
(77) PURRMANN, R.: U. S. patent 2,345,215.
(78) PURRMANN, R., AND MAAS, M.: Ann. **556,** 186 (1944).
(79) SACHS, F., AND MEYERHEIM, G.: Ber. **41,** 3957 (1908).
(80) SCHÖPF, C.: Naturwissenschaften **28,** 478 (1940).
(81) SCHÖPF, C.: Naturwissenschaften **30,** 359 (1942).
(82) SCHÖPF, C., AND BECKER, E.: Ann. **507,** 266 (1933).
(83) SCHÖPF, C., AND BECKER, E.: Ann. **524,** 49 (1936).
(84) SCHÖPF, C., BECKER, E., AND REICHERT, R.: Ann. **539,** 156 (1939).
(85) SCHÖPF, C., AND KOTTLER, A.: Ann. **539,** 128 (1939).
(86) SCHÖPF, C., KOTTLER, A., AND REICHERT, R.: Ann. **539,** 168 (1939).
(87) SCHÖPF, C., REICHERT, R., AND RIEFSTAHL, K.: Ann. **548,** 82 (1941).
(88) SCHÖPF, C., AND WIELAND, H.: Ber. **59,** 2067 (1926).
(89) SIMMONS, R. W., AND NORRIS, E. R.: J. Biol. Chem. **140,** 679 (1941).
(90) STOKSTAD, E. L. R.: J. Biol. Chem. **149,** 573 (1943).
(91) TARTTER, A.: Z. physiol. Chem. **266,** 130 (1940).
(92) TOTTER, J. R.: J. Biol. Chem. **154,** 105 (1944).
(93) TOTTER, J. R., AND DAY, P. L.: J. Biol. Chem. **147,** 257 (1943).
(94) TOTTER, J. R., MIMS, V., AND DAY, P. L.: Science **100,** 223 (1944).
(95) TOTTER, J. R., SHUKERS, C. F., KOLSON, J., MIMS, V., AND DAY, P. L.: Federation Proc. **2,** 72 (1943).
(96) TOTTER, J. R., SHUKERS, C. F., KOLSON, J., MIMS, V., AND DAY, P. L.: J. Biol. Chem. **152,** 147 (1944).
(97) TRAUBE, W.: Ann. **432,** 266 (1923).
(98) TSCHESCHE, R., AND WOLF, H. J.: Z. physiol. Chem. **244,** I-III (1936).
(99) TSCHESCHE, R., AND WOLF, H. J.: Z. physiol. Chem. **248,** 34 (1937).
(100) WEIJLARD, J., TISCHLER, M., AND ERICKSON, A. E.: J. Am. Chem. Soc. **67,** 802 (1945).
(101) WEYGAND, F.: Osterr. Chem. Ztg. **44,** 254 (1941).
(102) WIELAND, H., AND DECKER, P.: Ann. **547,** 180 (1941).
(103) WIELAND, H., AND KOTZSCHMAR, A.: Ann. **530,** 152 (1937).
(104) WIELAND, H., AND LIEBIG, R.: Ann. **555,** 146 (1944).
(105) WIELAND, H., METZGER, H., SCHÖPF, C., AND BÜLOW, M.: Ann. **507,** 226 (1933).
(106) WIELAND, H., AND PURRMANN, R.: Ann. **539,** 179 (1939).
(107) WIELAND, H., AND PURRMANN, R.: Ann. **544,** 163 (1940).
(108) WIELAND, H., AND SCHÖPF, C.: Ber. **58,** 2178 (1925).
(109) WIELAND, H., AND TARTTER, A.: Ann. **543,** 287 (1940).
(110) WIELAND, H., TARTTER, A., AND PURRMANN, R.: Ann. **545,** 209 (1940).
(111) WIGGLESWORTH, V. B.: Proc. Roy. Soc. (London) **B97,** 149 (1924).
(112) WILSON, H. E., SASLAW, S., AND DOAN, C. A.: J. Lab. Clin. Med. **31,** 631 (1946).
(113) WRIGHT, L. D., AND SKEGGS, H. R.: Proc. Soc. Exptl. Biol. Med. **55,** 92 (1944).
(114) WRIGHT, L. D., AND WELCH, A. D.: Science **98,** 179 (1943).
(115) ZUELZER, W. W., AND OGDEN, F. N.: Proc. Soc. Exptl. Biol. Med. **61,** 176 (1946).