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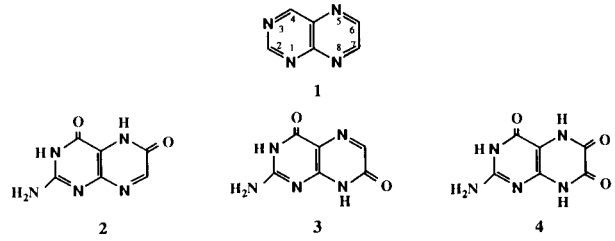
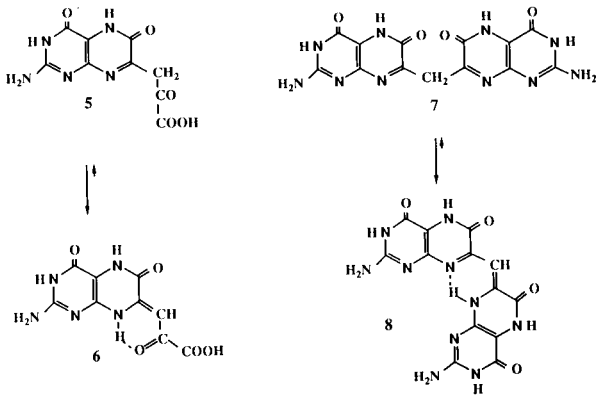
The origin of pterdine chemistry is associated with wing pigments of butterflies studied for the first time by Frederick Gowland Hopkins more than 100 years ago. His first publications in 1889 [1] described the isolation of the yellow pigment from the common English brimstone butterfly, and six years later he isolated a white pigment from the cabbage butterfly [2]. From 1924 to 1926 these pigments were further purified by Clemens Schöpf [3,4] in Heinrich Wieland's laboratory and have been named according to their colors and appearence in nature xanthopterin (**2**) and leucopterin (**4**). In 1933 during the course of this work a third component, isoxanthopterin (**3**) [5], was isolated but its constitution remained also unknown until Robert Purrmann was able to devise and perform logical syntheses [6-8] of all three compounds which turned out to be derivatives of the pyrazino[2,3-*d*]-pyrimidine ring-system termed by Wieland [9] "pteridine" (**1**).

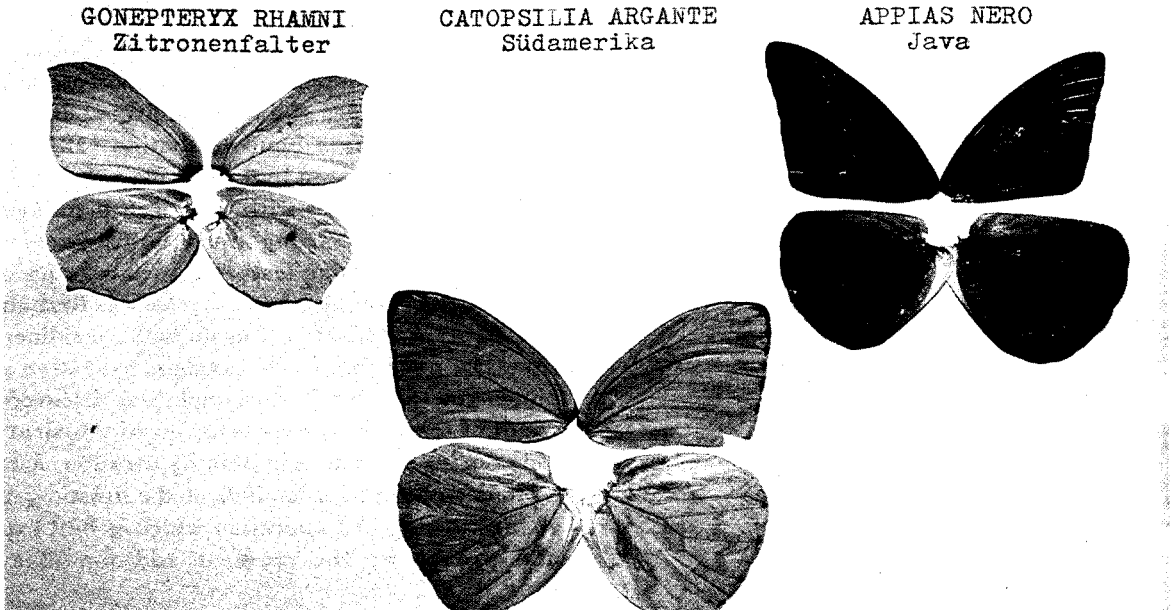
The period of 15 years of investigations on the structures of the butterfly pigments indicates already some principal difficulties encountered with pteridines which are attributed to their incomplete combustibil-

Scheme 1



Scheme 2





ity, high melting and decomposition points, poor solubilities in water and most organic solvents and hence problems in purification. More detailed investigations with tropical butterflies from South America and Java revealed two more deeply coloured pigments, the orange-red erythropterin (**6**) [10] and the violet pterorhodin (**8**) [11].

These structures demonstrate very nicely the importance of the correct tautomeric forms accounting for the observed physical properties of the molecules. Both compounds would be colourless if the side chain of erythropterin (**5**) would not have tautomerized to a hydrogen-bounded vinylogous amide as did also the original dixanthopterylmethane structure **7** in pterorhodin. We also should keep in mind that the thermodynamically favoured tautomers of amino- and hydroxy-substituted nitrogen-heterocycles are represented by the amino and lactam configuration, respectively. Six-membered nitrogen-heterocycles, of which the pteridine nucleus can be regarded as a prototype of a π-deficient ring system, reveal in general unexpected solubility properties which are contrary to the common observations with aliphatic and aromatic compounds.

Insertion of hydrophilic amino, hydroxy and thiol groups into nitrogenous heteroaromatics decrease solubility in water with increasing number of those substituents. Especially strong effects are observed in the presence of both an amino and an hydroxy group in the same molecule as documented in the wing pigment of butterflies. Gradual insertion of 1-4 hydroxy groups into the pteridine ring lowers the solubility to 1:58000 which is also found for 2-amino-4(*H*)-pteridone whereas isoxanthopterin and leucopterin are even 4 and 12 times less soluble, respectively.

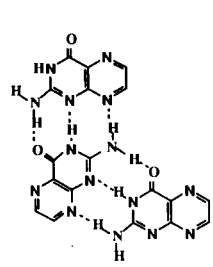
Table 1

SOLUBILITIES IN WATER

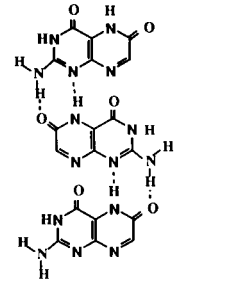
| SUBSTANCE | 20° | 100° |
|---|---|---|
| **Pteridine** | 7 | < 0.2 |
| 2-Hydroxy- | 600 | 50 |
| 4-Hydroxy- | 200 | 29 |
| 2.7-Dihydroxy- | 1 400 | 100 |
| 4.6-Dihydroxy- | 5 000 | 300 |
| 2.4.7-Trihydroxy- | 12 000 | 1 400 |
| 4.6.7-Trihydroxy- | 27 000 | 7 000 |
| 2.4.6.7-Tetrahydroxy- | 58 000 | 7 000 |
| 2-Methoxy- | 80 | 4 |
| 3-Methyl-4-oxo-3.4-dihydro- | 70 | 9 |
| 2-Mercapto- | 2 600 | 420 |
| 2-Methylmercapto- | 320 | 40 |
| 2-Amino- | 1 350 | 100 |
| 2-Methylamino- | 320 | 35 |
| 2-Dimethylamino- | 2 | |
| 2.4-Diamino- | 3 000 | 130 |
| 4.6.7-Triamino- | 12 500 | 450 |
| 2.4.6.7-Tetraamino- | 13 000 | |
| 2-Amino-4-hydroxy- | 57 000 | |
| 2-Acetamino-4-hydroxy- | 450 | |
| 2-Amino-4.6-dihydroxy- | 40 000 | |
| 2-Amino-4.7-dihydroxy- | 200 000 | |
| 2-Amino-4.6.7-trihydroxy- | 750 000 | |

To explain these results crystal-lattice forces, of a strength uncommonly high for organic substances, are brought into play by hydrogen-bonding between the amino, hydroxy, and thiol groups on one hand and the ring-nitrogen atoms on the other. This leads to intermolecular aggregation since the hydrophilic groups exert even more attraction for another than they do for the molecules of water.

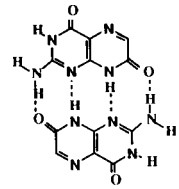
Scheme 3



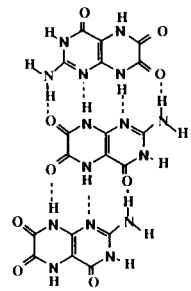
PTERIN



XANTHOPTERIN



ISOXANTHOPTERIN



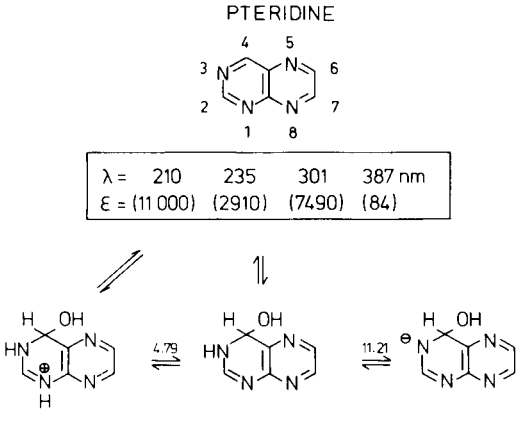
LEUCOPTERIN

The weightiest evidence in support of this hypothesis comes from studies of *O*- and *N*-methyl derivatives, since blocking of the active hydrogen by an hydrophobic methyl group causes a large increase in solubility as a consequence of a reduced probability of intermolecular hydrogen-bond formation [12]. Another correlation is also seen in the melting points, which are higher for pteridines with hydrogen-bonding substituents strongly stabilizing crystal-lattice forces.

Another interesting feature associated with a series of π-deficient nitrogen heterocycles was first observed in 1951 by A. Albert [13] again with pteridines which show the phenomenon of covalent hydration of C=N bonds [14,15]. The first example was discovered as a result of the very curious behavior of 6-hydroxypteridine during p*K*-determination by titration. A hysteresis loop on back titration indicated a drastic structural change during this operation which is best explained and proven by the covalent addition of a water

molecule across the 7,8-positions [16]. Anomalous p*K*a values, unusual UV, IR and NMR spectra often point to a covalent hydration as indicated, for example, with pteridine. In aqueous solution pteridine reveals in the normal *p*H range a basic p*K*a of 4.21 which is much too high in comparison to pyrimidine and pyrazine, and even an acidic p*K*a of 11.21 which is not at all in agreement with the basic structure of the molecule. Covalent hydration across the 3,4 C=N bond explains both facts since the adduct makes a formal dihydro species of higher basicity and known acidic character.

Scheme 4




Xanthopterin is also partially covalently hydrated as a neutral species as seen from its UV spectrum which shows a relatively low extinction of the long wavelength absorption band and a shoulder at 310 nm indicating a 7,8-dihydro derivative.

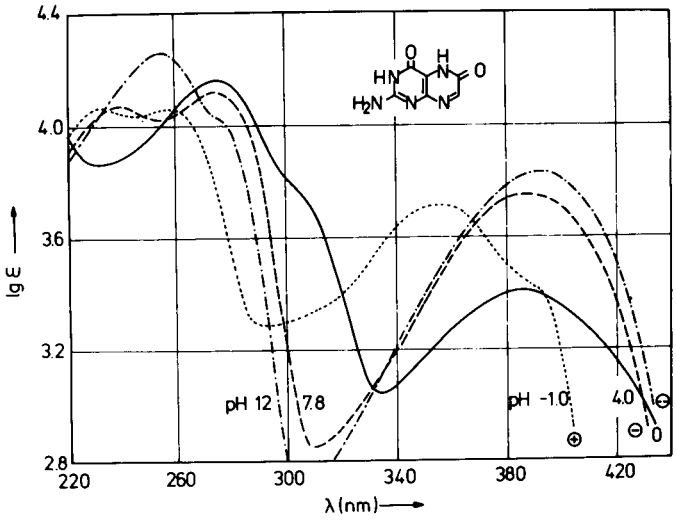
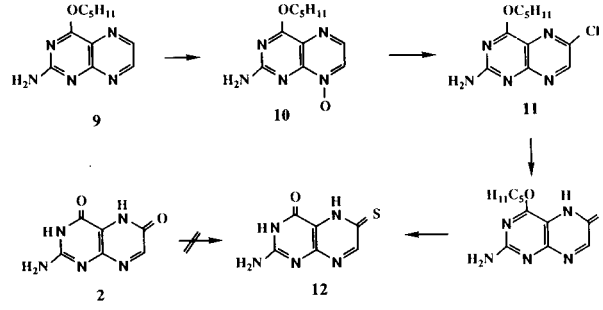



Figure 1

Addition of methanol to an aqueous solution of xanthopterin shifts the equilibrium even further towards adduct formation which is compared after 6 hours. 5-Methylxanthopterin equilibrates much faster in methanol and the initial yellow color derived from the long wavelength absorption disappeared completely within 2 hours.

Also 6-thioxanthopterin (**12**) which has recently been synthesized for the first time [17] from 2-amino-4-*n*-pentyloxypteridine (**9**) *via* its 8-oxide **10** and the rearranged 6-chloro derivative **11**, was studied regarding its behavior in adduct formation.

Scheme 5




The UV spectra of the various molecular species like cation, neutral form, mono- and dianion, however, did not indicate any anomalies pointing to any type of covalent hydration despite the fact that the sulfur atom is responsible for a lower basic and consequently higher acidic character of this molecule.

Adduct formation has furthermore been observed with 8-substituted lumazine and pterin derivatives [18,19], respectively. 3.8-Dimethyllumazine, for example, reveals an "acidic" p*K*a of 10.41 which is consistent with pseudobase formation in the 7-position and not due to any proton ionization as usual. This anion formation is associated with an unusually strong hypsochromic shift of the spectra from 398 nm of the neutral form to 308 nm in the monoanion. On the other hand, cation formation causes also a blue shift since protonation takes place at the N-1 atom shorting the merocyanine chromophore by partial localization of π-electrons. The equilibria and spectra are shown in Figure 5..

Examination of the corresponding 6,7,8-trimethyllumazine showed analogous behavior for the cation and the neutral form of the molecule whereas in basic medium a new type of monoanion must exist due to an UV-absorption at 362 nm. This is in agreement with a deprotonation from the activated 7-methyl group giving rise to an exo-methylene function as seen in the NMR spectrum [20] (Figure 6).

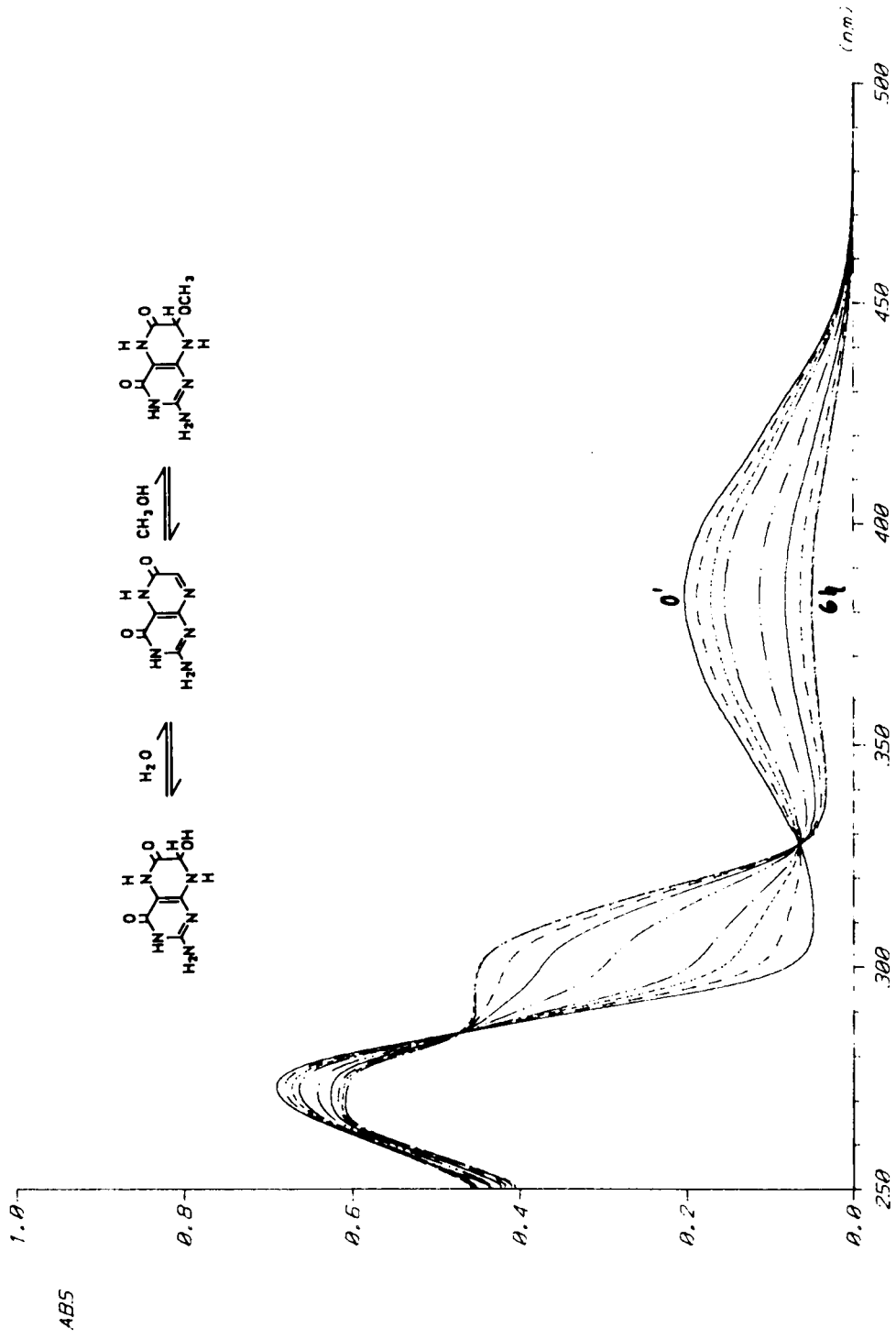

ABS
1.0
0.8
0.6
0.4
0.2
0.0
250
300
350
400
450
500
(nm)
H2O
CH3OH
OCH3
OH
6
6'

Figure 2

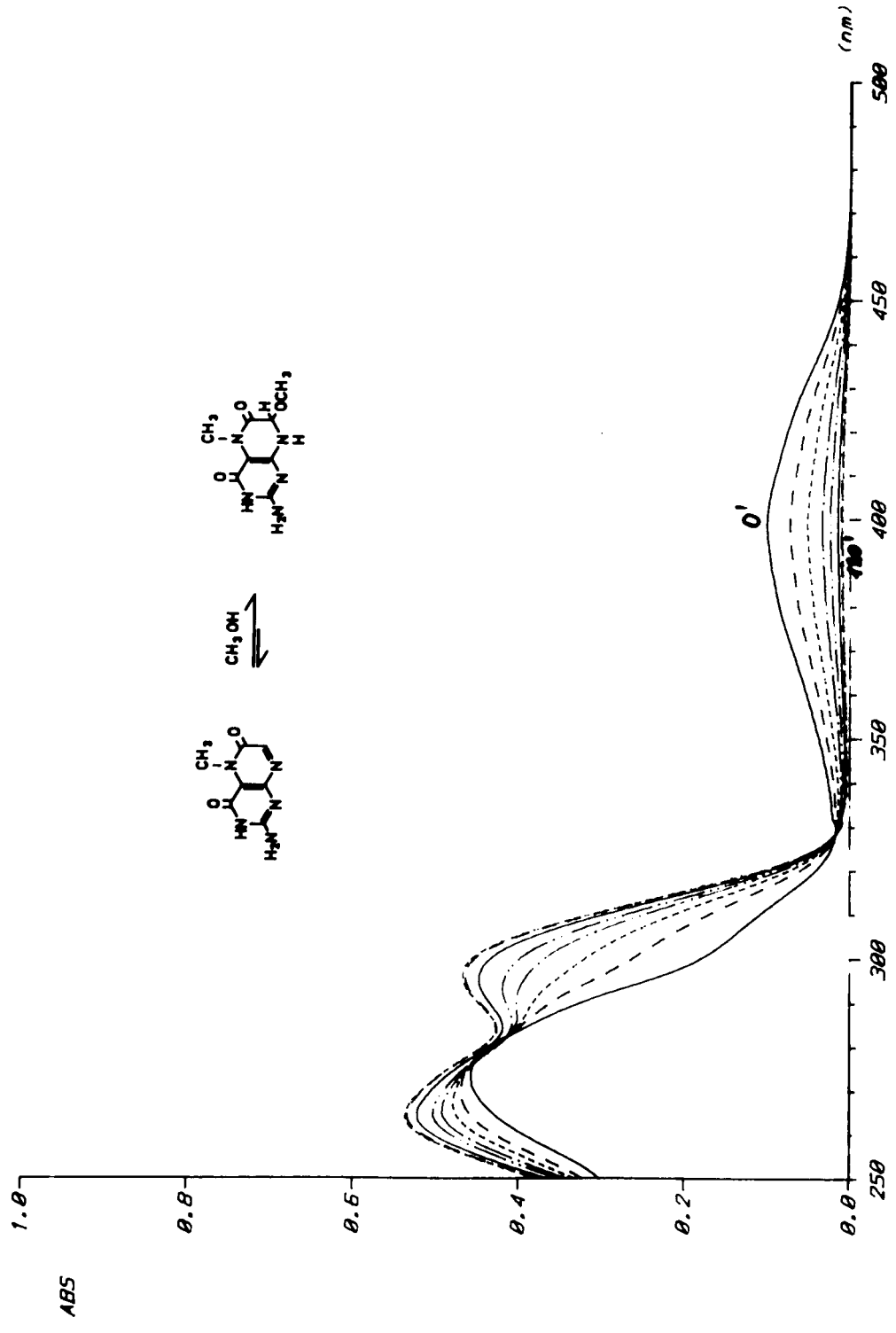



Figure 3

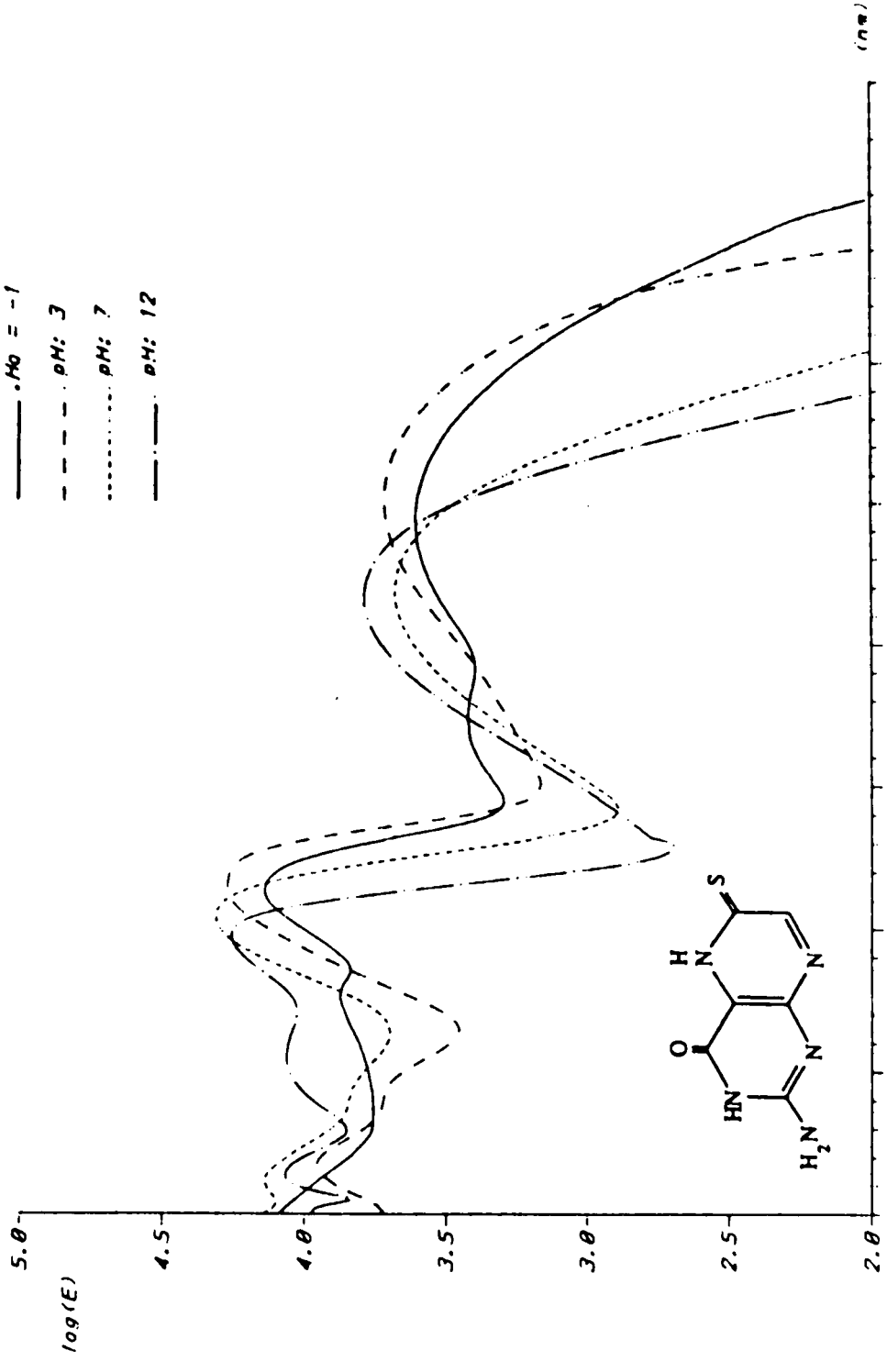

H0 = -1
pH: 3
pH: 7
pH: 12
log(E)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
200
250
300
350
400
450
500
550
600
(nm)
O
HN
H
N
S
N
N
N
H2N

Figure 4

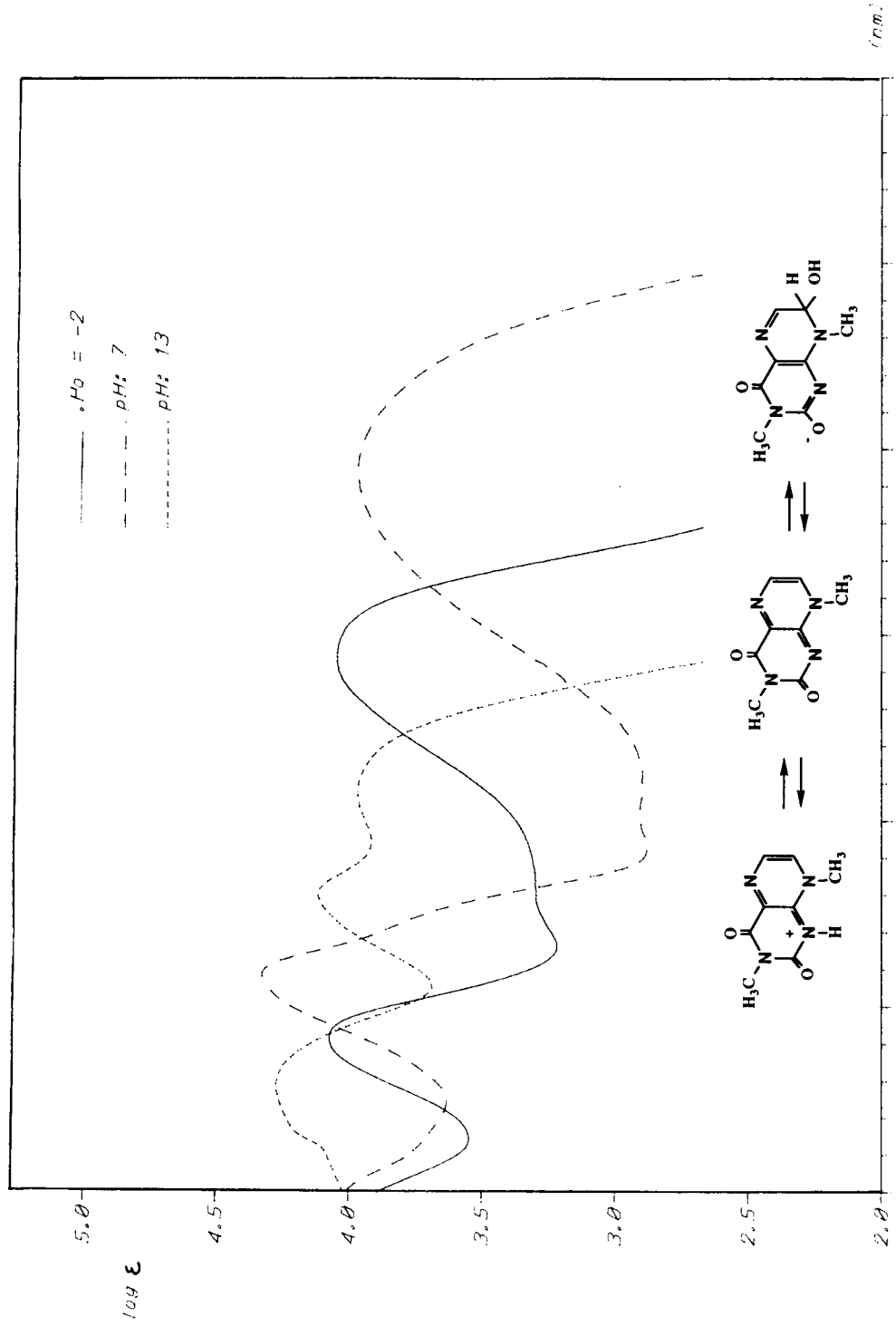
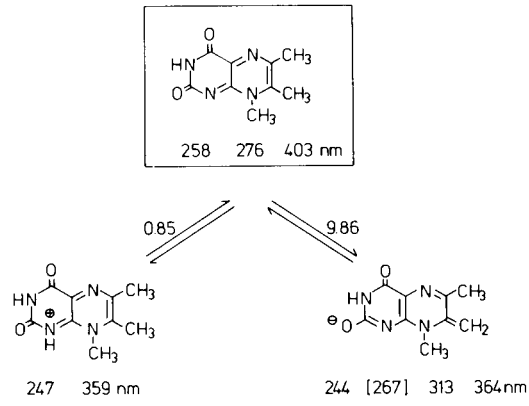

$H_0$ = -2
pH: 7
pH: 13
log ε
5.0
4.5
4.0
3.5
3.0
2.5
2.0
200
250
300
350
400
450
500
nm
$H_3C$
$CH_3$
H
OH
O
N
+
-

Figure 5




Another interesting example of the same series was found in the 6,7-dimethyl-8-ribityllumazine, an important intermediate in the biosynthetic pathway of riboflavin. This compound exhibits similar features in the acidic and neutral *p*H-region but anion formation is again of different nature due to a pronounced hypsochromic shift of almost 100 nm in the UV spectra and a somewhat lower acidic p*K*a-value of 8.29. These physical data are in accordance with intermolecular adduct formation of the β-hydroxy group of the ribityl residue to the C-7 position leading to an anion of a 7,8-dihydrolumazine species (Figure 7).

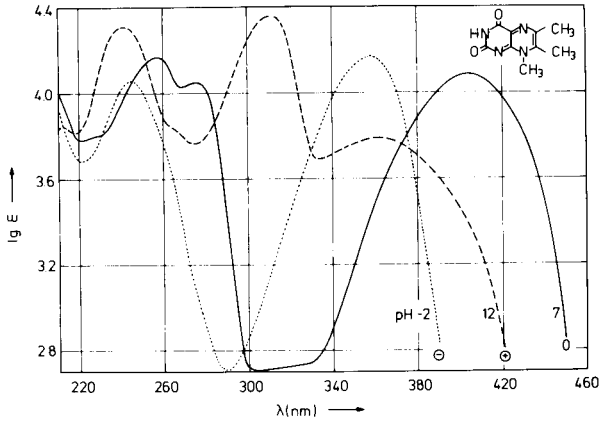



Figure 6

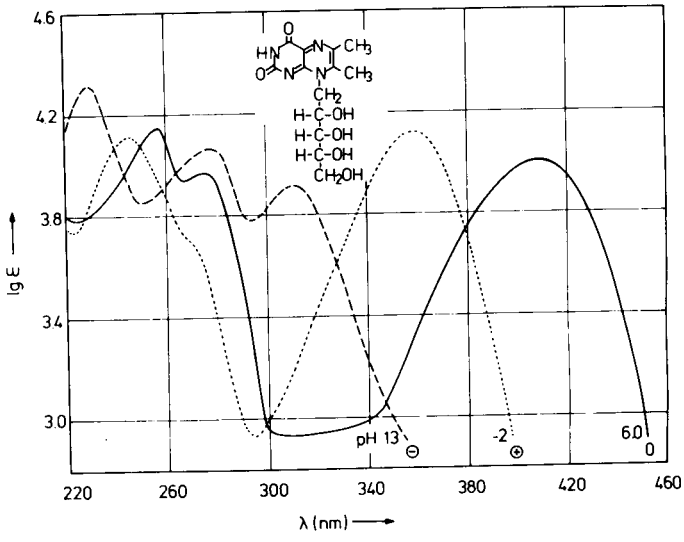
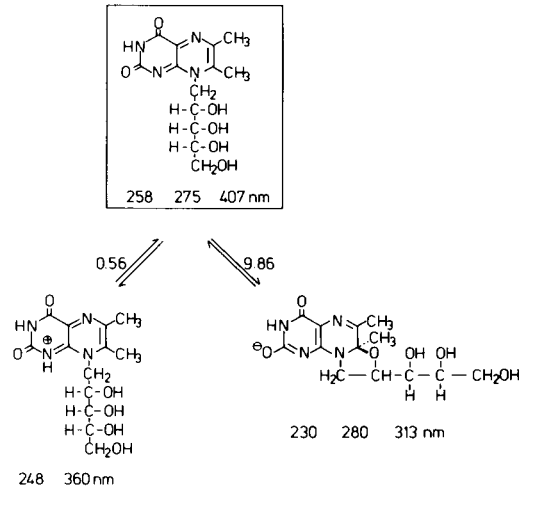



Figure 7



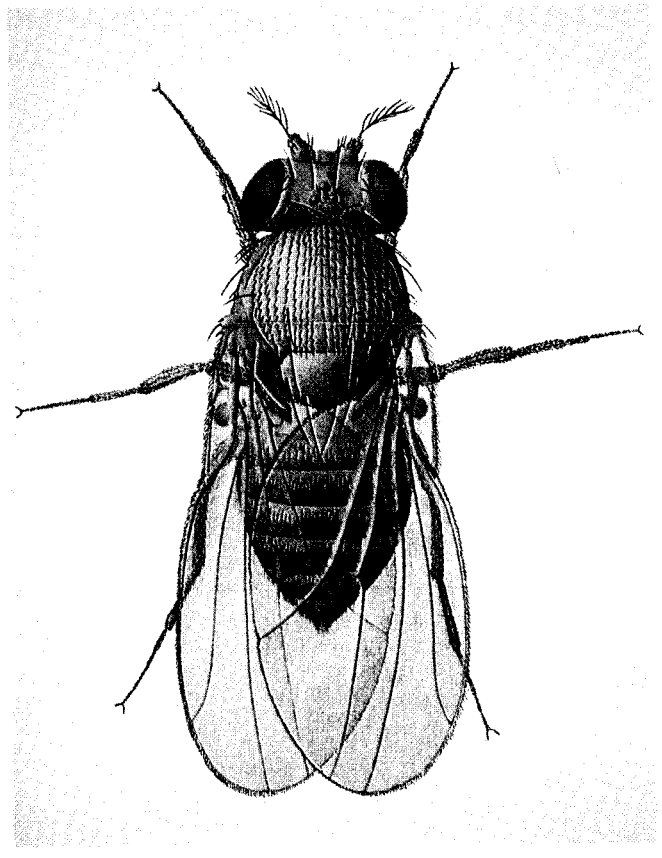



Figure 8

Talking about naturally occurring pteridines it has to be mentioned that a broad variety of compounds are very widely distributed in the plant and animal kingdom. Pterin [21] and lumazine derivates are found in higher plants and chloroplasts, in microorganisms, in insect, tunicata, amphibia, invertebrates, fish and reptiles as well as in mammals [22].

One of the most interesting structures derived from all the many investigations became obvious during the structural elucidation of the red eye pigments of the fruitfly *Drosophila melanogaster* which lasted another 20 years till this problem could be solved by us in 1975 [23]. The *Drosophila* is unfortunately a very tiny fly with deeply colored eyes from which the pigments have been isolated. Depending on the mutant pigmentation can, however, vary from almost colorless to yellow and even dark red.

Chromatography allowed the separation of the various pigments of which the red fraction consisted of drosopterin, isodrosopterin and neodrosopterin. Viscontini who investigated this problem for the first time noticed already that the former two components are not only isomeric but they are even enantiomers of unusual chiroptical properties as seen from their ORD- and CD-spectra (Figure 10).

After various suggestions of incorrect structures for the drosopterins (**13**) my coworker Norbert Theobald elucidated by a combination of modern NMR and mass-spectral techniques the following complicated constitution showing a formal dimeric pterin derivative in which one pyrazine moiety has been ring-extended to a 7-membered 1,4-diazepine ring [23].

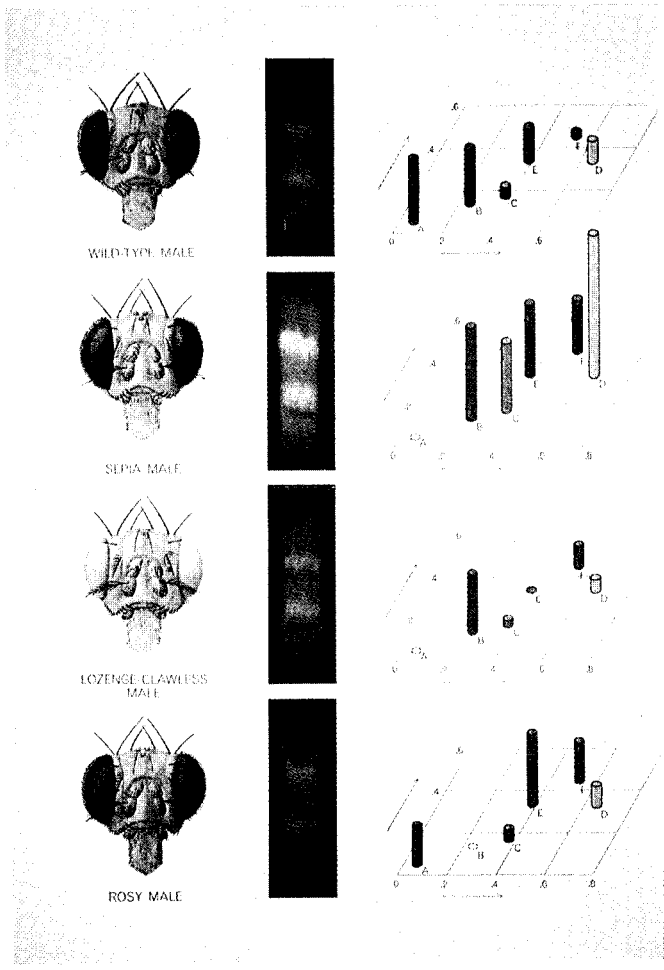



Figure 9

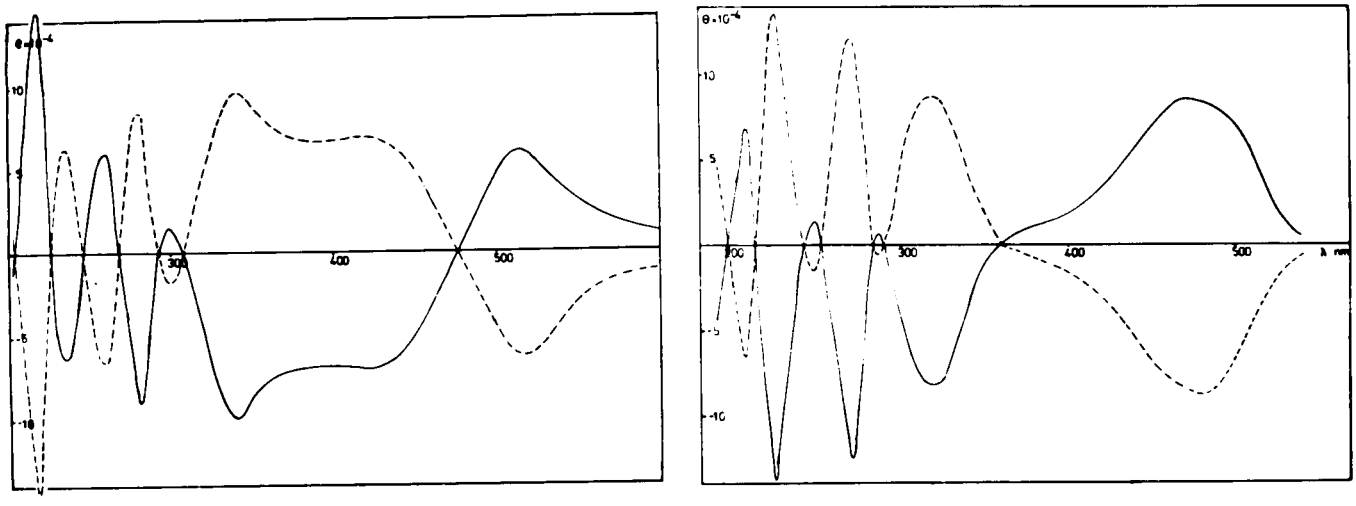
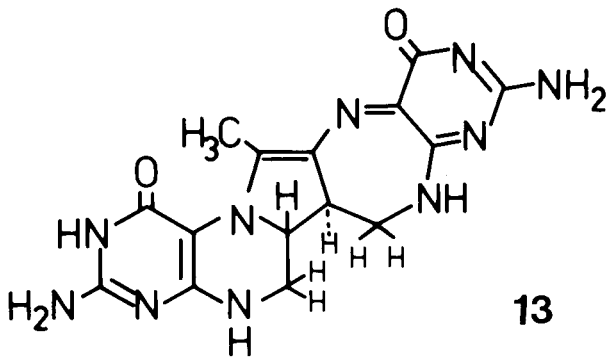


Figure 10



13

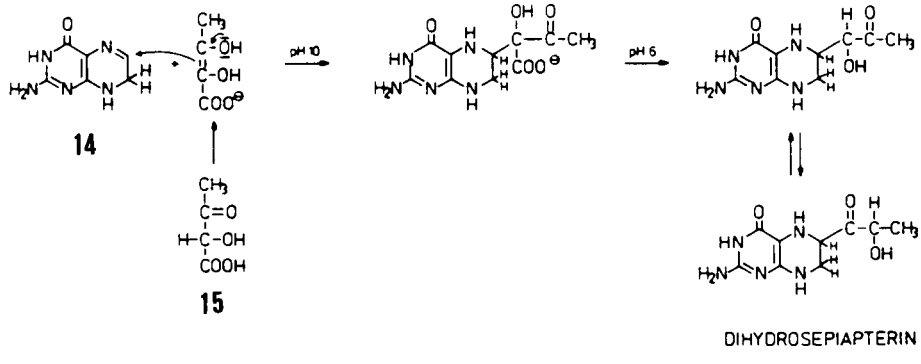
Formula

The most striking result was achieved by the fact that this complex structure could be synthesized in a one-pot reaction starting only from two components, 7,8-dihydropterin (**14**) and α-hydroxy-β-ketobutyric acid (**15**) under special *p*H conditions. The racemate was then obtained in a 37% yield. A proposed mechanism for the various steps of these interconversions is seen in the next Scheme 7 and 8:

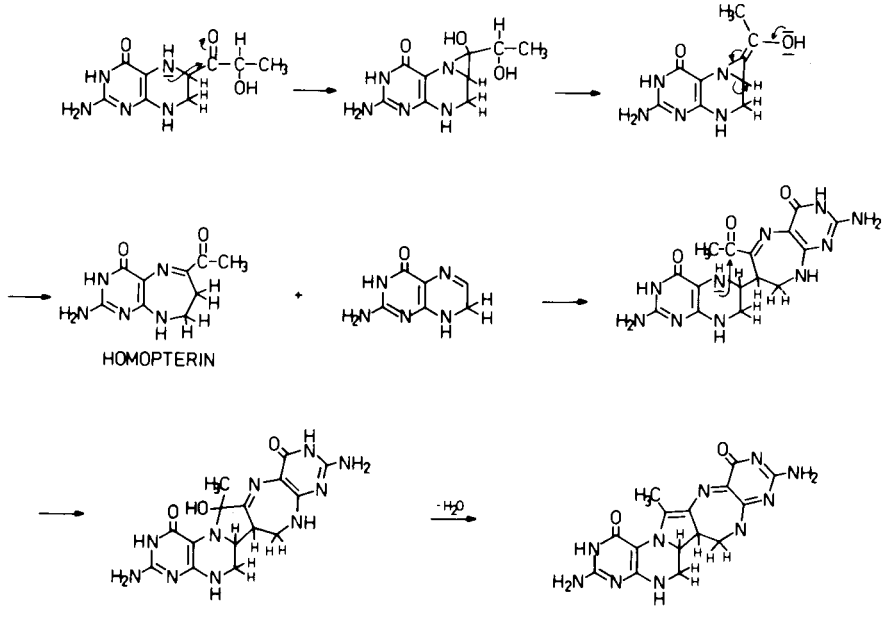
There are still some unsolved problems in this series like the stereochemistry of the chiral centers C-6 and C-6' as well as the structures of neodrosopterin and aurodrosopterin which are associated with the main pigments droso- and isodrosopterin. All attempts to crystallize either of these compounds have so far not been successful.

Another natural occurring pterin of unusual structure was found in *Euglena gracilis* by Elstner and coworkers and we have been able to elucidate chemical constitution of *Euglenapterin* as $N^2$,$N^2$-dimethyl-6-(L-threo-1,2,3-trihydroxypropyl)pterin (**16**) [24]. This is the first case where the 2-amino group has been further modified by methylation. During the structural elucidation of this substance *p*H-dependent UV-spectra gave us the first hint that the 2-amino group must have been derivatized due to the fact that the

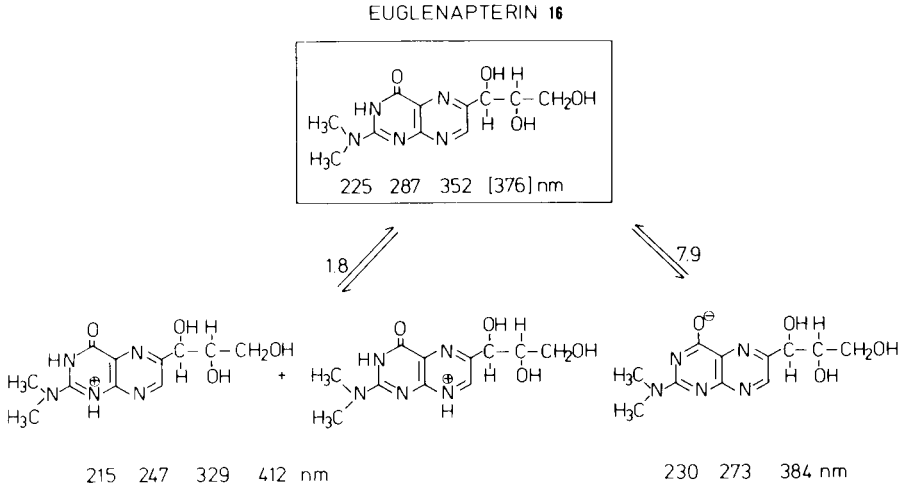
Scheme 7




Scheme 8



Scheme 9




cation form showed an additional absorption band in the visible region (Figure 11). According to model studies [25] this observation is consistent with a partial N-8 protonation from steric reasons forming a long cross-conjugated π-system.

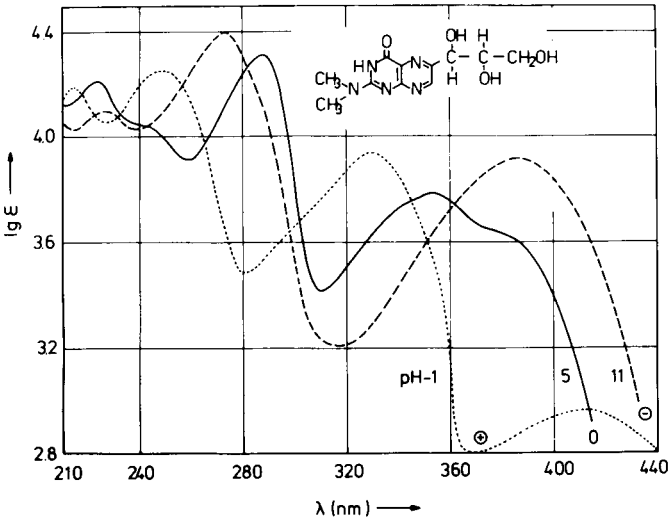



Figure 11

Photochemistry.

It is also a well-known fact that most of the pteridine derivatives are light-sensitive compounds. Work-up of natural material and isolations have to be done in a dark room to avoid photodegradations. We have studied more closely 8-substituted lumazines [26] and noticed that the ease of cleavage of the N-8 substituent depends on the nature of the functional groups at the β-position of the side-chain. The half life of the photodealkylation of 8-isopropyl-3,6,7-trimethyllumazine in methanol is 5 minutes, of 8-(β-hydroxyethyl)-3-methyllumazine 20 seconds (Figure 12) and of 8-(β-piperidinoethyl)-3-methyl-6,7-diphenyllumazine 8 seconds in acetonitrile, respectively.

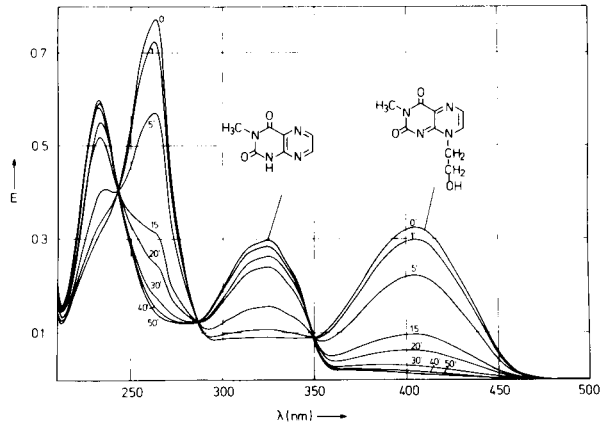
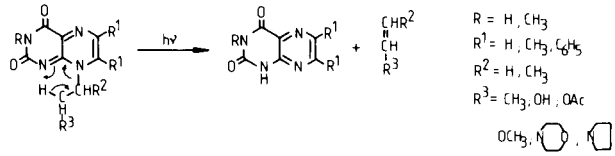



Figure 12

The mechanism of these photodegradations belongs to the Norrish-type II reactions where the N-1 atom initiates the intramolecular H-abstraction from the β-position of the side-chain. A six-membered cyclic

Scheme 10



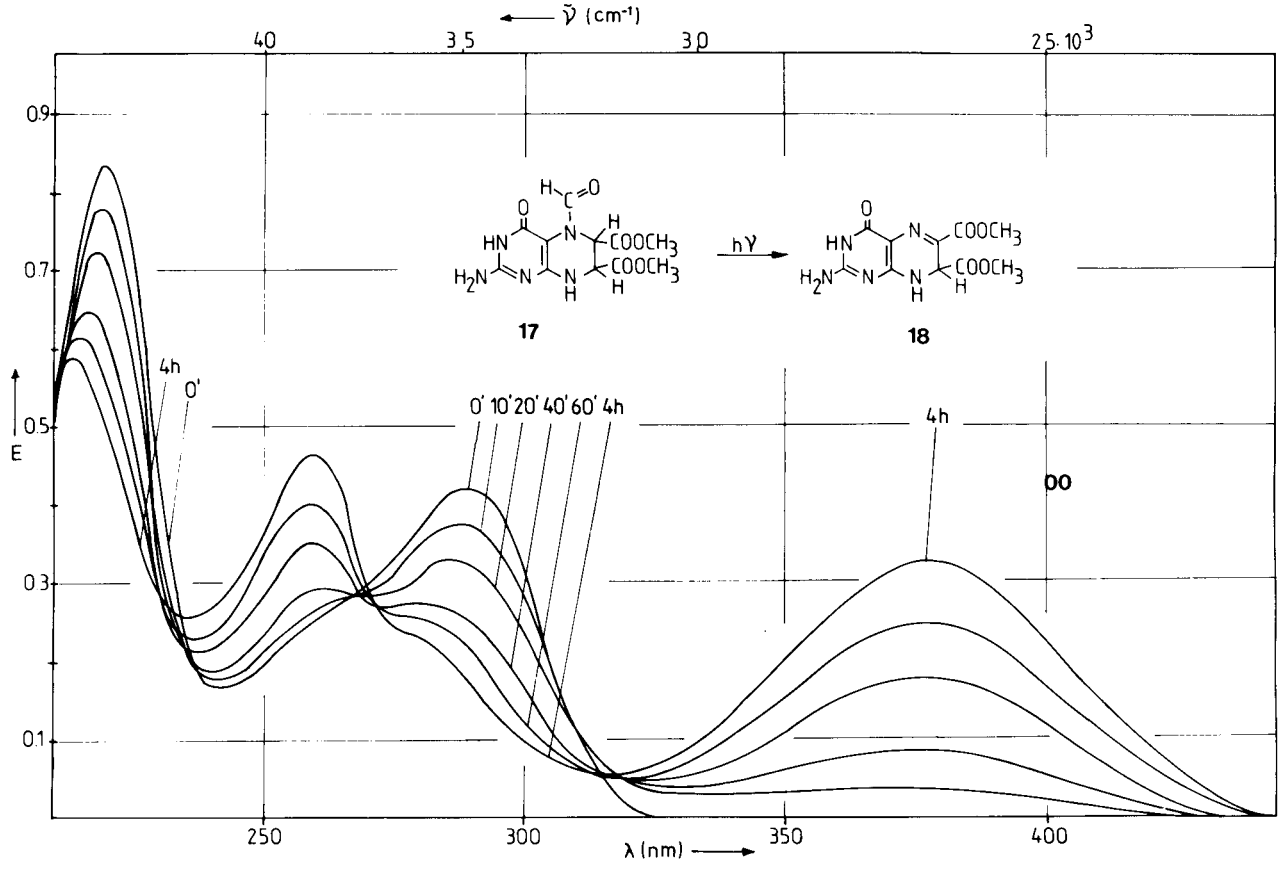
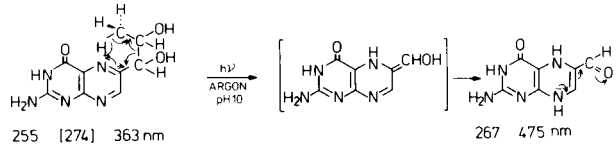



Figure 13

transition state facilitates this reaction to an intermediary 1,4-diradical which then undergoes the elimination to the lumazine derivative. In case of 8-β-hydroxyethyllumazines acetaldehyde which is derived from tautomerism of eliminated vinylalkohol, could be detected and is considered as proof of the proposed mechanism.

There are also some other examples of photocleavage reactions which fall into the same category of bond scissions. The methyl 5-formyl-5,6,7,8-tetrahydropterin-6,7-dicarboxylate (**17**) is converted into the methyl 7,8-dihydropterin-6,7-dicarboxylate (**18**) (Figure 13) [26] and irradiation of biopterin (**19**) under anaerobic conditions in basic medium (*p*H 10) resulted in a Norrish-type II cleavage forming the red-colored 5,8-dihydropterin-6-carboxaldehyde (**21**). This unusual structure reveals an antiaromatic 8-π-system in the pyrazine moiety which attains its stability by the electron-attracting formyl group inducing a vinylogous amide resonance in the molecule. This interesting type of pterin derivative is derived from its transient 6-hydroxymethylene form **20** which tautomerize to the 5,8-dihydro structure. On admission of oxygen spontaneous dehydrogenation to pterin-6-carboxaldehyde takes place.

Scheme 11




The same type of C-C bond cleavage is also observed with 7-isobiopterin (**22**) leading to 5,8-dihydropterin-7-carboxaldehyde (**23**) according to analogous structural arguments (Figure 14).

There is furthermore a strong *p*H-dependence in the photochemistry of pteridines since sepiapterin shows a dehydrogenation under anaerobic conditions at *p*H 0 (Figure 15) whereas a more severe breakdown involving the side-chain occurs at *p*H 5-12 and leading to pterin-6-carboxylic acid [27] (Figure 16).

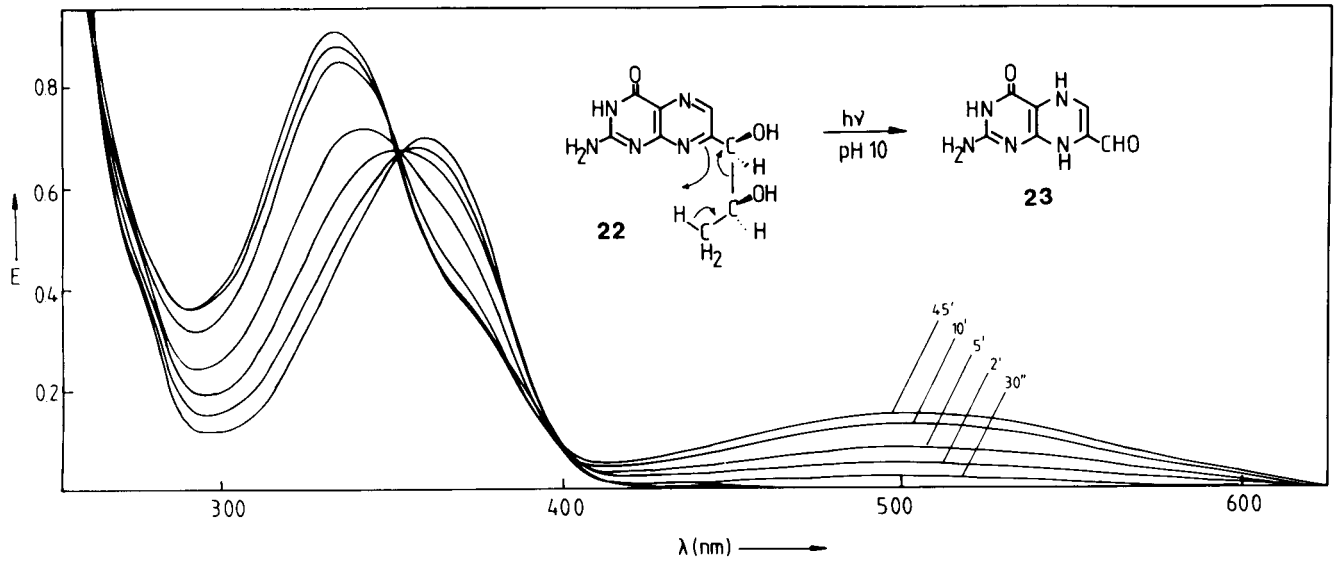



Figure 14

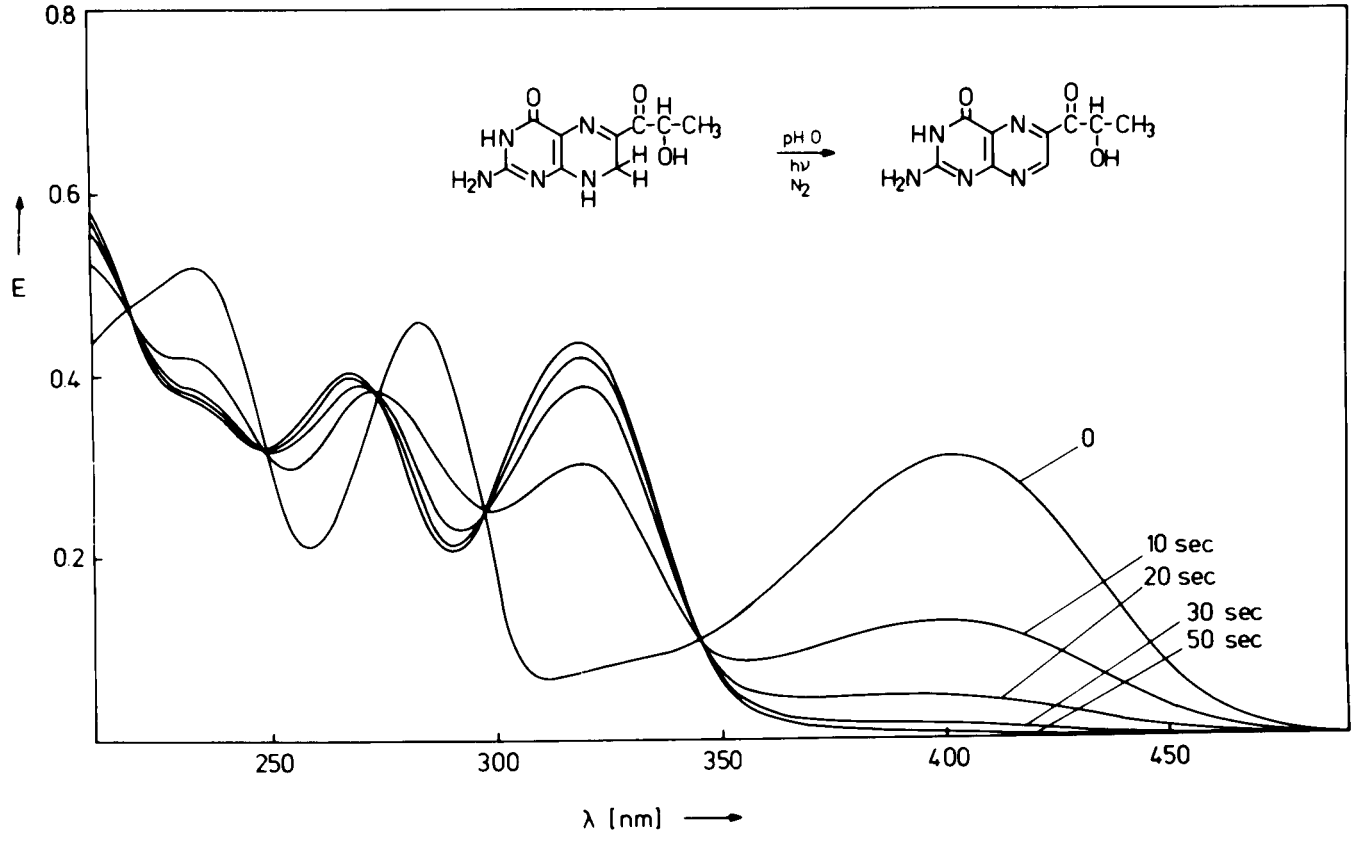



Figure 15

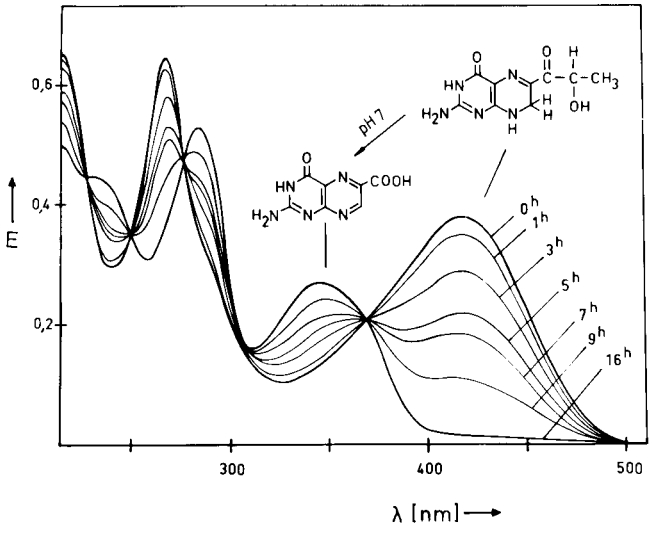



Figure 16

Photodecarboxylation was observed with 1,3-dimethyllumazine-7-carboxylic acid (Figure 17) but not with the 6-isomer and long irradiation of $N^2$,$N^2$,7-trimethylpterin at *p*H 9 resulted even in an *N*-demethylation (Figure 18).

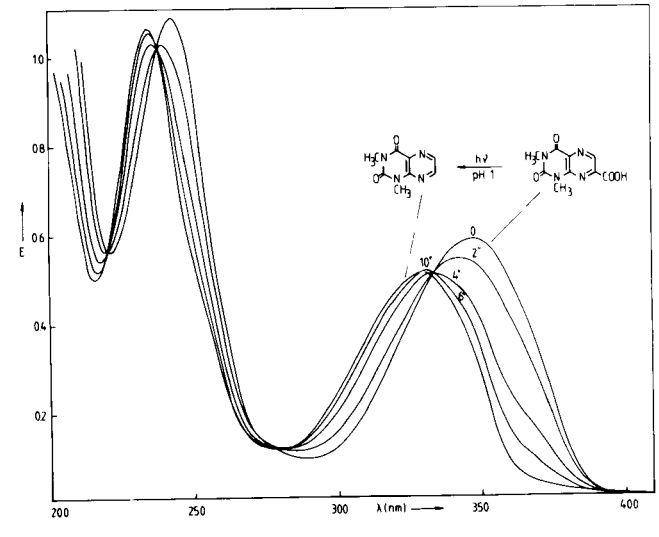



Figure 17

Electrochemistry.

Pterdines, in general, appear to be readily reduced electrochemically due to the π-deficient character of this heterocyclic ring system. Reduction takes place mainly in the pyrazine ring giving rise to various dihydro- and tetrahydro derivatives of more or less biological significance. Our interest in electrochemical reductions of various pteridines traces back to the observation that the electrolysis of leucopterin in

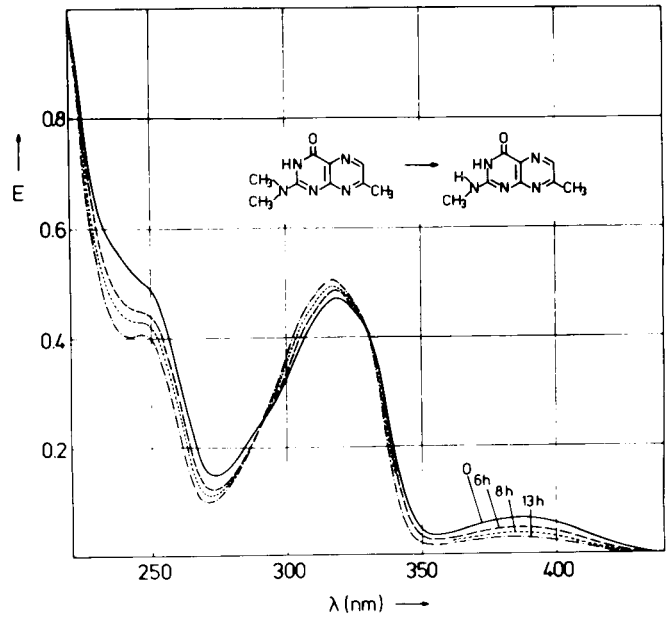
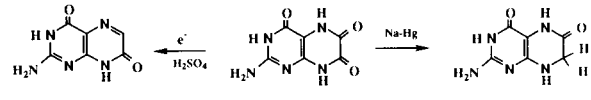



Figure 18

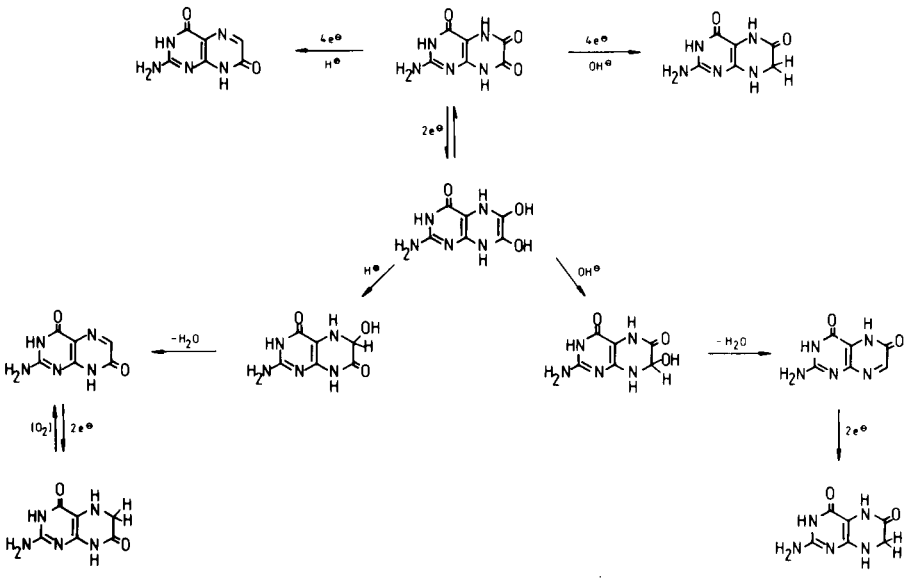
strong acidic medium leads to isoxanthopterin whereas the chemical reduction with sodium amalgam in basic solution afforded 7,8-dihydroxanthopterin.

Scheme 12




Systematic electrochemical studies with leucopterin and its 3- and 8-methyl derivatives over a wide *p*H-range indicated that there is an interesting *p*H-dependence of the reduction process leading to an isoxanthopterin derivative at low *p*H and a xanthopterin derivative at higher *p*H, respectively. Since 4 electrons are consumed in both cases electrochemically by the substrate we propose a general mechanism for the initial $2e^-/2\ H^+$ step involving the redox-active 6,7-dioxadiene system to form an endiol as the first most likely intermediate. This labile compound will then tautomerize according to the *p*H into two different covalent hydrates which after elimination of one mole of water to isoxanthopterin and xanthopterin, respectively, will immediately be further reduced to the corresponding 5,6- and 7,8-dihydro derivatives. On work-up the easily oxidizable 5,6-dihydroisoxanthopterin is then converted into the heteroarmatic form, whereas the much more stable 7,8-dihydroxanthopterin can be isolated directly in its reduced stage.

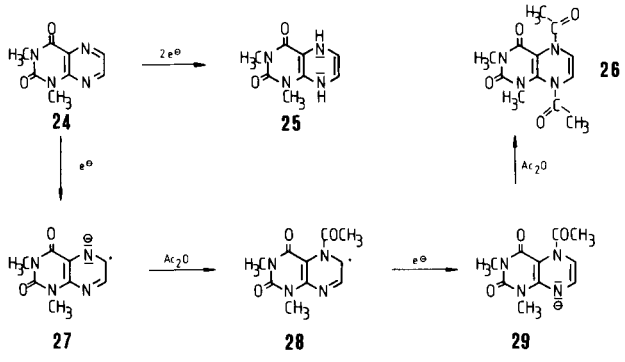
Scheme 13



6,7-Dioxotetrahydrolumazine and its 1,3-dimethyl derivate follow the same *p*H-dependent electrochemical pathway of leucopterin, but various other 2- and/or 4-substituted 6,7-dioxotetrahydropteridines behave differently and react in acidic and basic solution to the corresponding 7,8-dihydro derivatives [28]. The characteristic structural feature of leucopterin and its analogues is the diamide configuration in the pyrazine moiety putting in this way two carbonyl functions in adjacent positions and forming a formal 1,4-dioxadiene system. This part is electrochemically active and has its counterpart in other 1,4-diheterodiene combinations, the simplest of which can be seen in the pyrazine ring itself revealing a 1,4-diazadiene structure.

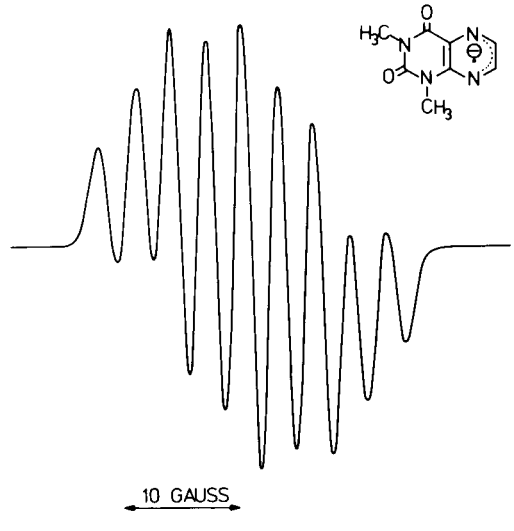
1,3-Dimethyllumazine (**24**) is expected under these aspects to be reduced in a $2e^-/2H^+$-step to the corresponding 5,8-dihydro derivate **25**. This form is thermodynamically unstable due to the antiaromatic 8 π-electron character and could therefore not be isolated as a solid. Its formation as an intermediate was, however, proven by trapping experiments with acetic anhydride yielding 5,8-diacetyl-5,8-dihydrolumazine (**26**), the constitution of which has been elucidated unequivocally by an X-ray analysis [29].

Scheme 14



The initial step of such an electrochemical reduction is in principle a one-electron transfer reaction giving rise to a radical anion **27**, which could be detected by ESR during electrolysis in dry DMF with tetra-*n*-butylammonium iodide as the electrolyte (Figure 19).

Figure 19

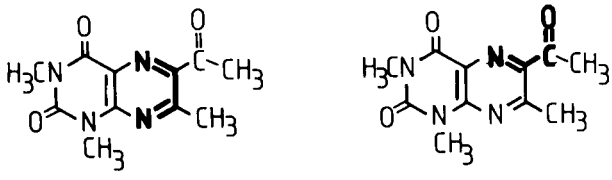



Compound **27** is a strong nucleophile reacting with acetic anhydride to give **28**, which will accept another

electron to the enamide anion **29** for the final reaction with a second acetic anhydride molecule to give the end-product **26**.

Another interesting system of potential electrochemical activity is represented by 6- and 7-acylpteridine derivatives. 6-Acetyl-1,3,7-trimethyllumazine (**30**) reveals a 1,4-diaza- as well as a 1,4-oxazadiene structure for a two electron-attack and formation of dihydro derivatives.

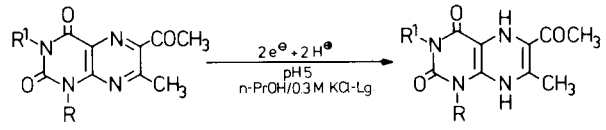
Scheme 15



**30**

6-Acyllumazines are electrochemically reduced at *p*H 5 in 1-propanol/0.3 *M* potassium chloride solution to the corresponding 5,8-dihydro derivatives which are stable enough under anaerobic conditions to be isolated as red solids. The acyl group counteracts the special 8π-electron system by its electron-attracting power and stabilizes this configuration by mesomeric interaction. The long wavelength absorption band of low extinction at 480-490 nm is very characteristic for this type of compounds as seen already during the photodegradation of biopterin.

Scheme 16



| R | $R^1$ |
|---|---|
| $CH_3$ | $CH_3$ |
| $CH_3$ | H |
| H | $CH_3$ |

| R | $R^1$ | $\lambda_{max.}$ |
|---|---|---|
| $CH_3$ | $CH_3$ | 488 |
| $CH_3$ | H | 488 |
| H | $CH_3$ | 485 |

The cathodic reduction of **30** in acidic medium (0.5 *N* hydrochloric acid/1-propanol) afforded the 6-acetyl-7,8-dyhydro-1,3,7-trimethyllumazine presumably through an acid-catalysed tautomerism of the 5,8-dihydro species. Again a different result was obtained during electrolysis in 1-propanol/0.3 *N* potassium chloride at *p*H 8-10 starting with 1,3-dimethyllumazine-6-carboxaldehyde (**31**) and the 7-carboxaldehyde **32** in an one electron reduction process and in a highly stereoselective manner to *threo*-glycol formation.

In a similar manner 7-hydroxy-1,3-dimethyllumazine-6-carboxaldehyde reacted to give the corresponding glycol which turned out to be base sensitive forming 2-oxo-5-methylamino-4-*N*-methylcarboxamido-1,2-dihydropyrazine-3-carboxaldehyde in a slow reaction at *p*H 13.

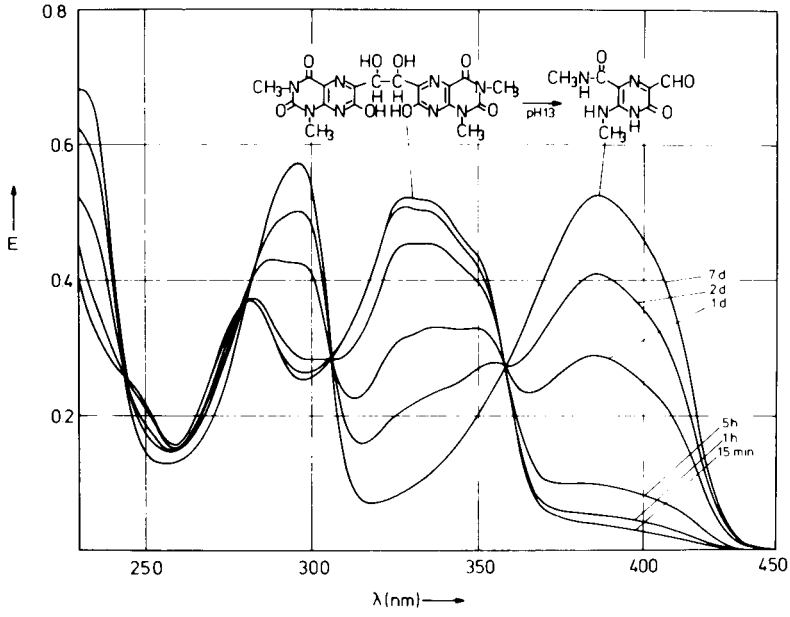
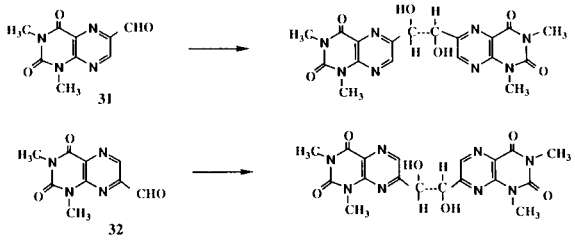



Figure 20

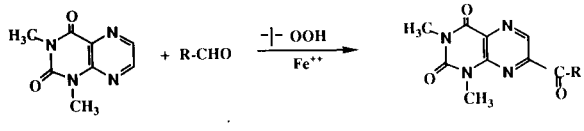
Scheme 17



The isomeric 1,3-dimethyl-6-oxo-5,6-dihydrolumazine-7-carboxaldehyde is reacting under analogous conditions in a $3e^-/3H^+$-reduction process to a glycol with simultaneous reduction of the C=N bond in the pyrazine moiety. Mild oxidation in weakly basic medium (*p*H 9) degrades this molecule to 1,3-dimethyl-5-oxo-5,6-dihydrolumazine and glyoxal (Figure 21).

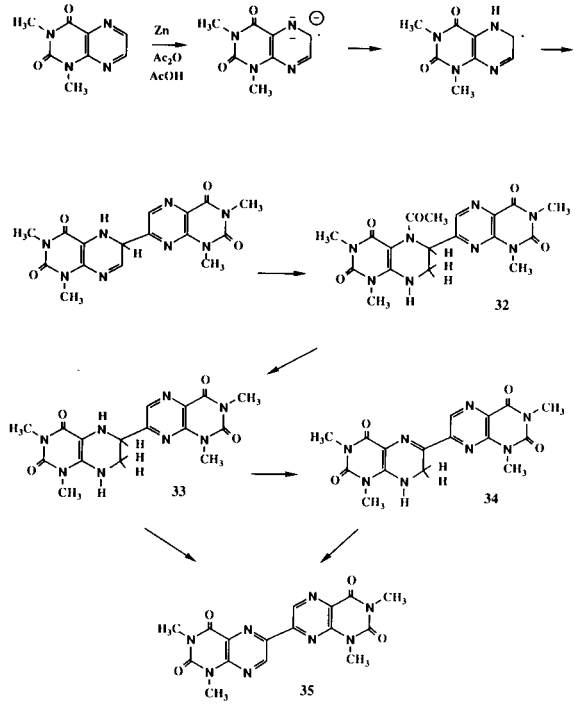
The electrochemical results encouraged us to investigate also the chemical reduction of the pteridine ring-system regarding a direct dimerization *via* the pyrazine moieties. Pteridines are prone to homolytic nucleophilic substitutions by acyl radicals, for example, to form preferentially 7-acyl derivatives [30].

Scheme 18



It was assumed that dimeric lumazines may be obtained by chemical means. Treatment of 1,3-dimethyllumazine with zinc in acetic anhydride/acetic anhydride led to a yellowish solution from which 5-acetyl-5,6,7,8-tetrahydro-6-(1,3-dimethyllumazin-7-yl)-1,3-dimethyllumazine (**32**) could be isolated in 50% yield.

Scheme 19



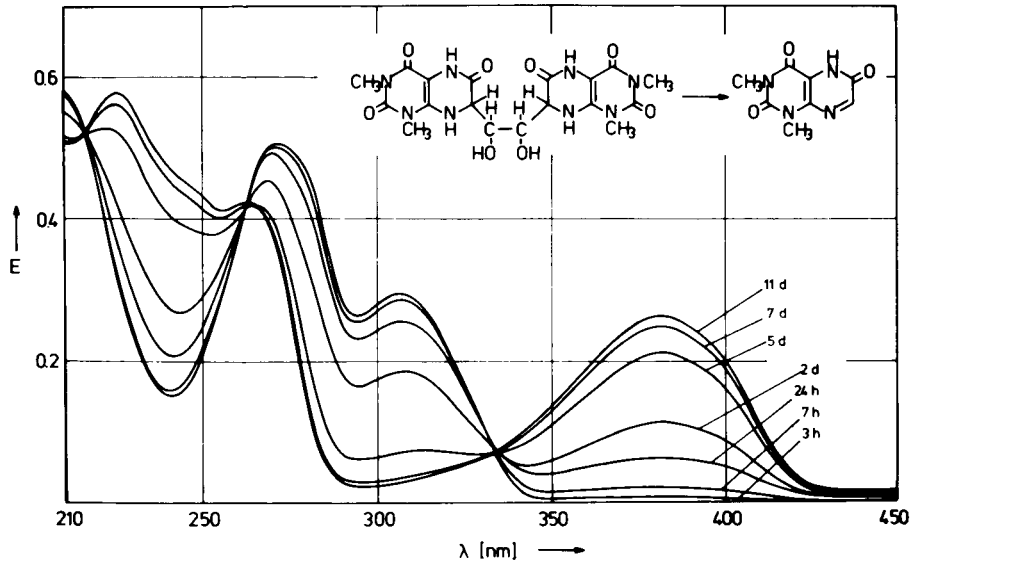
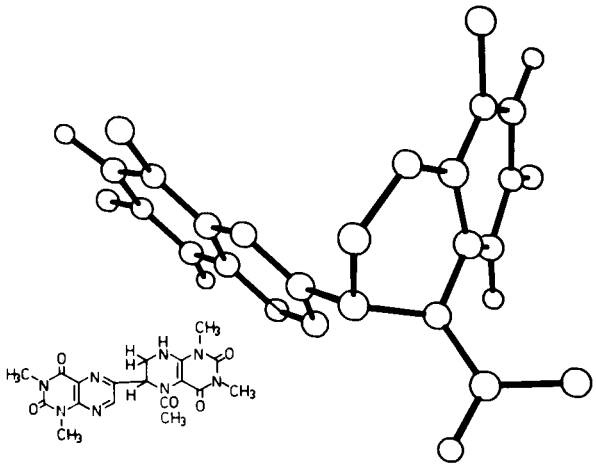



Figure 21

This structure is derived from the fact that the initially formed radical anion is attacking from its 6-position a second 1,3-dimethyllumazine species at the most π-deficient 7-position in a Minisci-type homolytic nucleophilic substitution connecting both nuclei in an unsymmetrical manner. Further reduction of the former nucleophile to the tetrahydro stage and subsequent acetylation leads obviously to a stable product. An X-ray analysis of this material confirmed the proposed structure.

Scheme 20



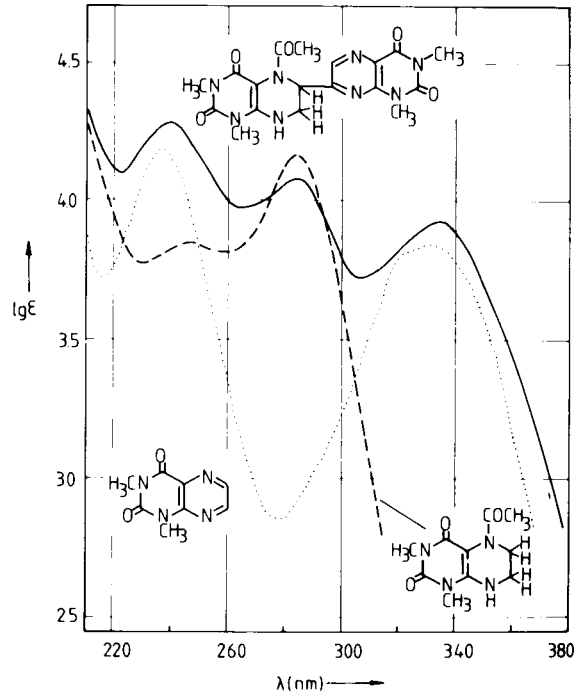



Figure 22

Deacylation can be achieved by methanolic hydrogen chloride to yield **33** which on air oxidation is converted to a stable, red-colored 7,8-dihydrobislumazinyl derivative **34**. Its color is due to the merocyanine chromophore extending through both lumazine moieties. Further oxidation by permanganate led to 6-(1,3-dimethyllumazin-7-yl)-1,3-dimethyllumazine (**35**). The UV/VIS spectra of the various type of lumazine dimers are summarized in Figures **22** and **23** and illustrate nicely the different oxidation stages of these unusual molecules.

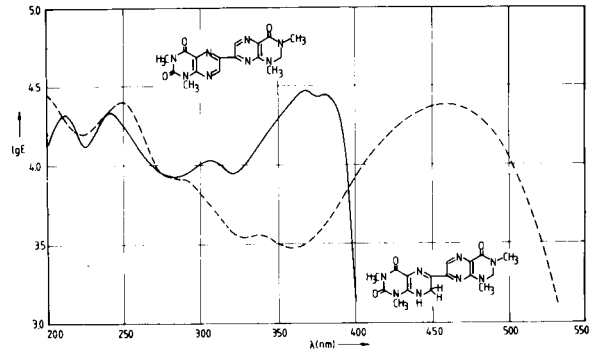


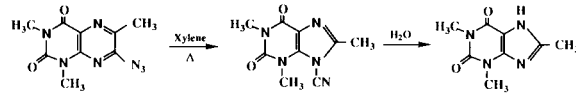

Figure 23

Ring Transformations.

Another interesting field of heterocyclic chemistry, in general, is dealing with ring transformation reactions which have also been observed with pteridines in special cases. Mainly the pyrazine moiety is involved showing preferentially ring contractions to purines but also ring enlargement reactions to condensed diazepine ring-systems. Our interest in such studies arose from earlier work of Huisgen [31] who could show that phenylazide rearranges in presence of nucleophiles thermally and photolytically to substituted azepines. Azido-*N*-heterocycles in contrast show usually ring contractions [32-35] initiated by the zwittazido cleavage reaction [36] as a common feature.

We first synthesized 7-azido-1,3,7-trimethyllumazine and obtained on heating in toluene expectedly, 9-cyano-1,3,8-trimethylxanthine which lost its cyano group easily on hydrolysis in water.

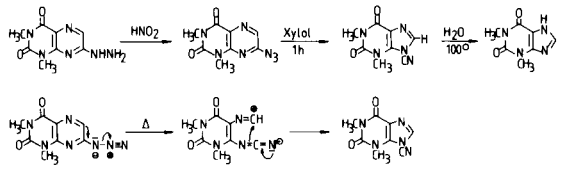
Scheme 21




7-Azido-1,3-dimethyllumazine was synthesized analogously either from 7-chloro-1,3-dimethyllumazine by nucleophilic displacement with sodium azide or by nitrous acid treatment of 7-hydrazino-1,3-dimethyllumazine under cooling and showed on prolonged

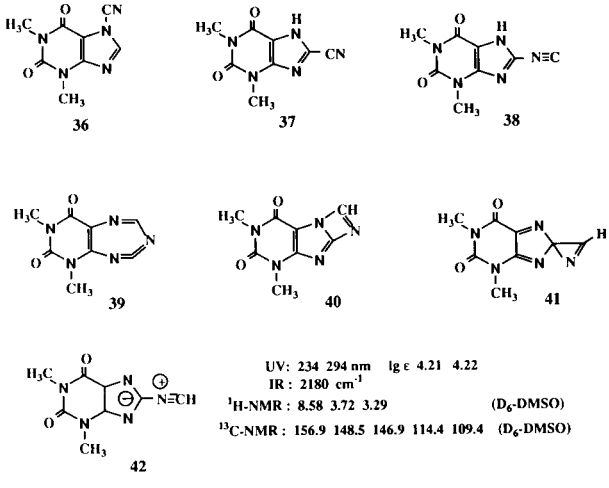
heating in principle the same ring contraction to 9-cyanotheophylline. It was, however, noticed that boiling in xylene forms a less soluble isomeric substance which precipitated from the hot solution.

Scheme 22



According to the physical data and its hydrolytic decomposition to 8-aminotheophylline this compound can neither be 7-**36** nor 8-cyanotheophylline (**37**), respectively. From the NMR spectrum in $D_6$-DMSO can further be concluded that we are also not dealing with theophyllin-8-yl isocyanide (**38**) due to a singlet signal at 8.58 ppm, which is rather a C-H than a N-H signal from its stability in deuterium oxide exchange experiments. Since heterocyclic azides show a broad variety of reactions the structures of a cyclic carbodiimide **39**, a condensed 1,3-diazeto[1,2-*f*]-purine derivative **40**, a spiro-compound **41** and a resonance stabilized nitrilium ylide **42**, respectively, have *a priori* to be considered.

Scheme 23

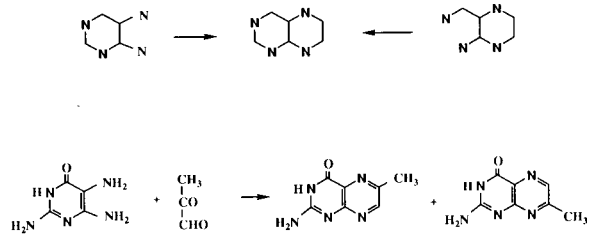



A characteristic band in the IR spectrum at 2180 $cm^{-1}$ excludes the possibilities of a tricyclic ring system so that the existence of a stable nitrilium ylide (**42**) is favoured [37]. A proof for this structure came from a gated decoupling experiment with the well-soluble *N*-(1-*n*-octyl-3-neopentylxanthin-8-yl)-nitrilium ylide which indicated in deuteriochloroform a $^1H$-$^{13}C$ coupling of 246 Hz at 145.5 ppm. These findings were to some extent very surprising since it was assumed thus far that a stable, crystalline nitrilium ylide [38] affords a bulky substituent at the nitrilium-C-atom to counteract decomposition. We believe that the unusual high thermodynamic stability of the new type of heterocyclic nitrilium ylides is due to a strong resonance stabilization of the negatively charged anion moiety of the molecule.

Pteridine Syntheses by Regioselective Condensations.

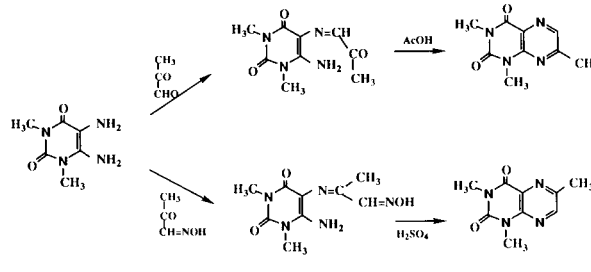
Pteridine syntheses can be achieved by two principle pathways starting either from an appropriately substituted pyrimidine by annelation of the pyrazine ring or *visa versa* by build-up of the pyrimidine ring from a pyrazine precursor. The most common approach uses the "Gabriel-Isay-condensation" [39] in reacting a 5,6-diaminopyrimidine with a 1,2-dicarbonyl compound.

Scheme 24



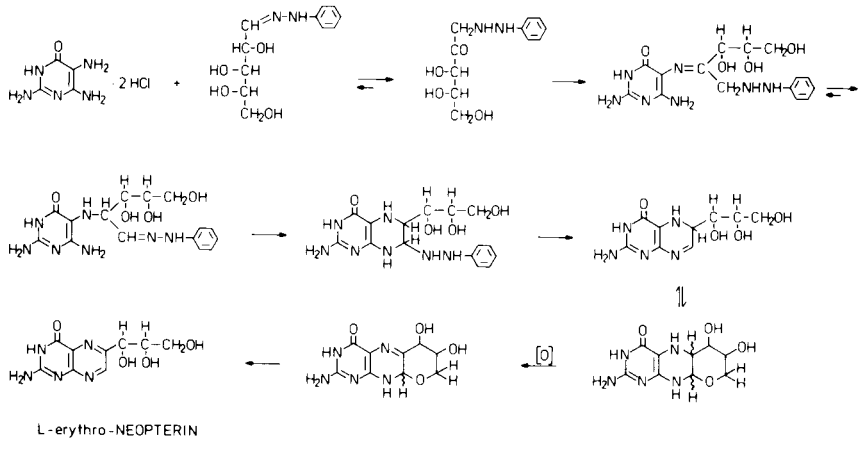
If the latter component, however, is unsymmetrical or an α-substituted monocarbonyl compound is applied, isomeric mixtures of the corresponding 6- and 7-substituted pteridine derivatives are usually obtained. Since their separation is in most cases difficult and time-consuming regioselective condensations like the Timmis-, Pachter- or Polonovski-Boon-reaction have been developed. We have recently found that even simple 6- or 7-alkylpteridine derivatives can be obtained by direct condensations in an almost regioselective manner if either the alkylglyoxal or its monoxime are reacted with the 5,6-diaminopyrimidine first to the intermediary Schiff's bases which cyclize in acetic and 80% sulfuric acid, respectively, in the expected manner.

Scheme 25



More difficulties have been encountered if carbohydrates are used in such condensation reactions leading always to complex mixtures of many reaction

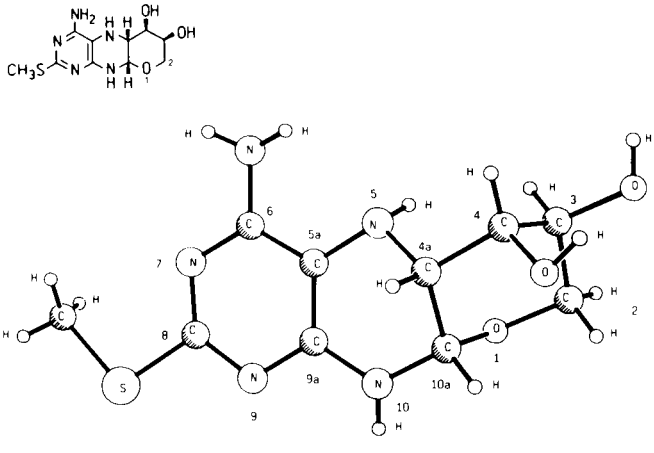
Scheme 26




products [40]. Under these circumstances a most striking result was obtained by Viscontini in his efforts to synthesize various naturally occurring pteridine derivatives carrying a 6-polyhydroxyalkyl side-chain. He succeeded in the synthesis of D-neo- and L-monapterin by a regioselective condensation between 2,5,6-triamino-6-pyrimidone dihydrochloride and the phenylhydrazones of D-xylose and L-arabinose, respectively, in high yields [41]. 5-Deoxy-L-arabinose was condensed under analogous conditions but the yield of biopterin did initially not exceed 10-15% and could later only be increased to 38% [42,43].

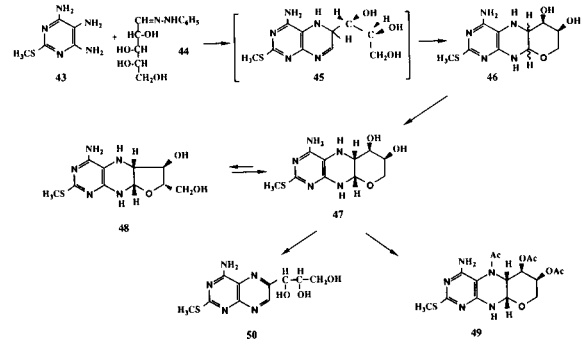
In order to explain these results we investigated the Viscontini reaction in more detail using 2-methylmercapto-,4,5,6-triaminopyrimidine (**43**) and L-arabinose phenylhydrazone (**44**) as a model [44]. Condensation under nitrogen atmosphere at 60° in acidic medium led to a diastereomeric mixture of pyrano[3,2-*g*]pteridine derivatives **46** from which one isomer **47** could be crystallized in pure form.

Scheme 27



Its X-ray structure determination is proving the correct stereochemistry. Furthermore is compound **46** another example of a stable intramolecular adduct derived from the 5,6-dihydropteridine precursor **45** by nucleophilic addition of the terminal OH-group of the side-chain. NMR studies with **46** are complex since an equilibrium mixture of the pyrano- (**47**) and furo[3,2-*g*]pteridine **48** isomers is established in $D_6$-DMSO. Mild acetylation of **46** gave a triacetate **49** and controlled oxidation by sodium iodate afforded 4-amino-6-(L-erythro-1,2,3-trihydroxypropyl)-2-methylmercaptopteridine (**50**) in 80% isolated yield. It can be concluded from these experiments that the oxidation of a formal tetrahydropteridine derivative is a straightforward process whereas conversion of a 5,6-dihydro species to the heteroaromatic oxidation level is associated with partial cleavage of the side-chain leading to low yields as found in biopterin synthesis.

Scheme 27a

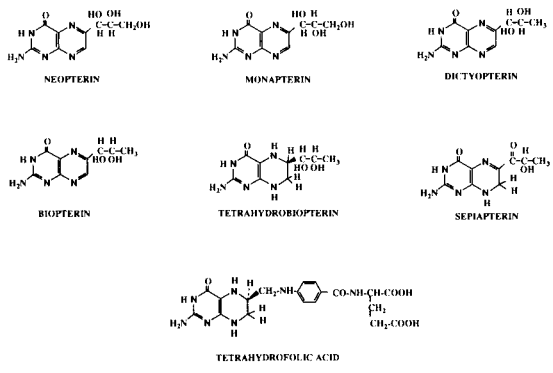


Biosynthesis of Pterdines.

Examination of the structures of many naturally occurring pteridines reveals a 2-amino-4-pteridone sub-

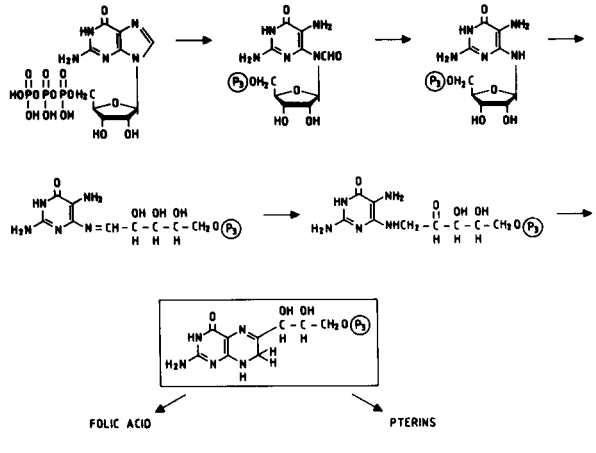
stituent pattern at the pyrimidine moiety and in most cases a carbon side-chain up to 3 C-atoms in the 6-position of the pyrazine ring.

Scheme 28



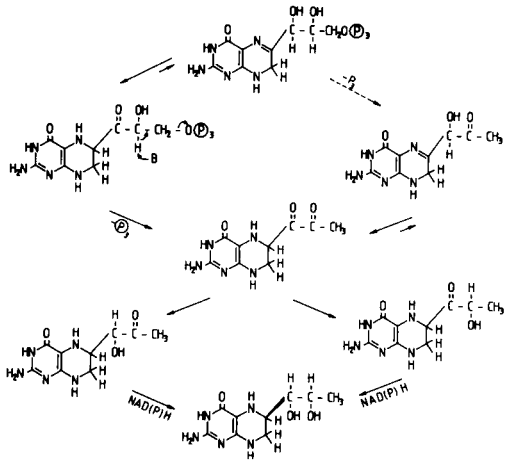

It was found that all natural pteridines are derived from the same precursor - guanosine-5'-triphosphate (GPT) (**51**) - which shows an interesting ring enlargement reaction due to the enzyme GTP-cyclohydrolyse catalysing the interconversion to 7,8-dihydroneopterin-3'-triphosphate (**52**) [45].

Scheme 29



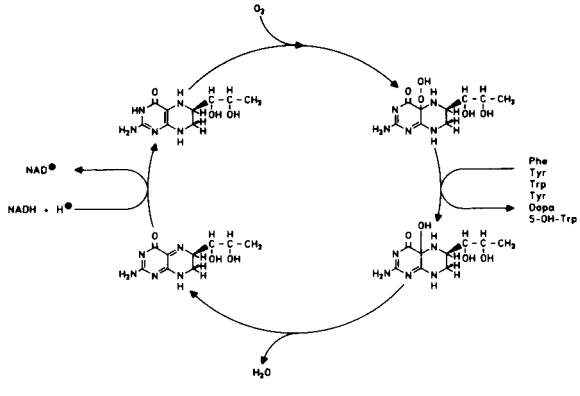

This compound can be regarded as the mother substance of all pterins the biosynthetic pathway of which has recently been elucidated in most details. For example the conversion of 7,8-dihydroneopterin-3'-triphosphate into 5,6,7,8-tetrahydrobiopterin (**54**) proceeds *via* tetrahydro-6-pyruvylpterin (**53**) in order to invert the stereochemistry of the chiral centers of the side-chain from D-*erythro* into L-*erythro* configuration.

Scheme 30




5,6,7,8-Tetrahydrobiopterin, on the other hand is a very important cofactor in hydroxylation reactions especially of aromatic compounds due to its ability to activate molecular oxygen for this purpose.

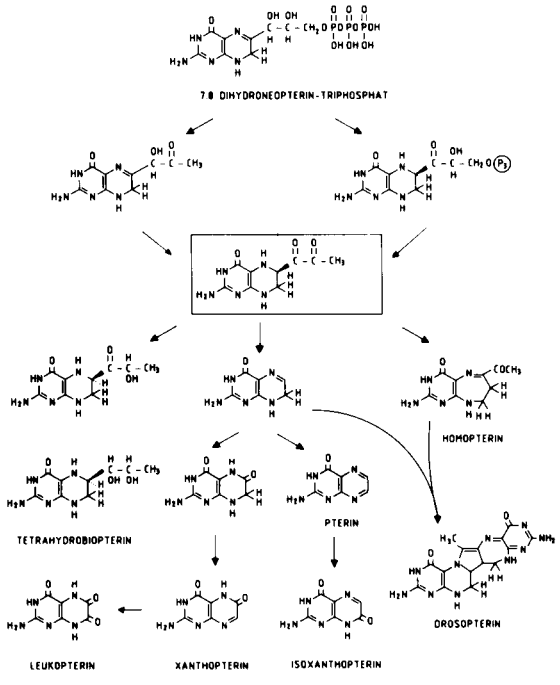
Scheme 31




Tetrahydro-6-pyruvylpterin (**53**) functions as a key intermediate in the biosynthetic transformations since a side-chain eliminating enzyme is cleaving off the C-6 pyruvyl residue to give 7,8-dihydropterin which is either further modified to the butterfly pigments xanthopterin, isoxanthropterin and leucopterin or reacts in a non-enzymatic reaction with homopterin to the racemic drosopterins. Homopterin has been detected by Jacobson [46] and independently characterized by us in a collaborative study [47] and by G. M. Brown and coworkers [48].

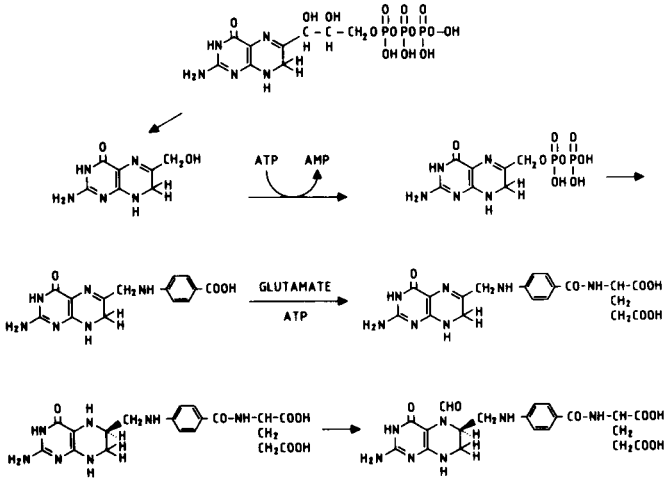
Finally it should be mentioned that folate and its derivatives are very important biologically active compounds which are involved in several metabolic areas, both biosynthetic and degradative pathways. Many of the reactions are common to most metabolically active

Scheme 32



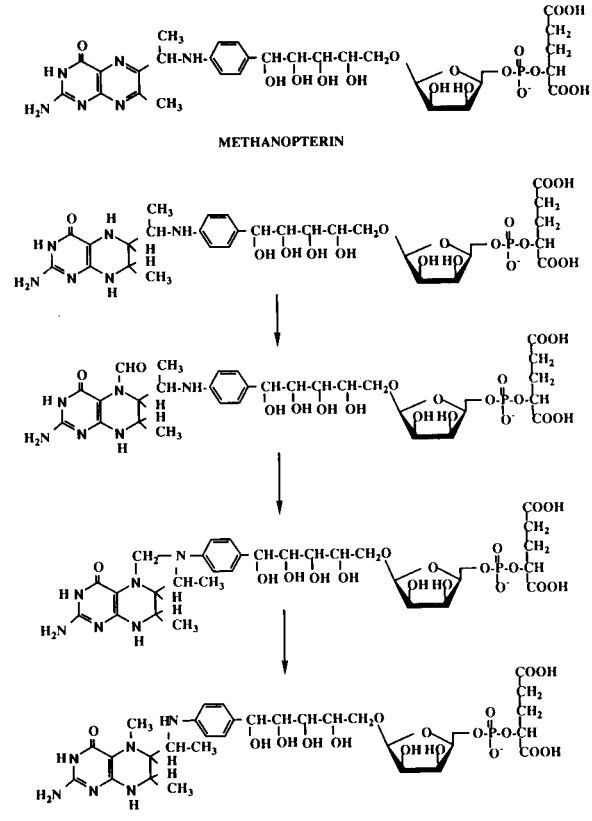

cells but there are various folate-dependent reactions that are species and/or tissue specific. Most important are the cofactor properties of 5,6,7,8-tetrahydrofolate in one-carbon transfer reactions which play a central role in purine-, thymidylate-, serine- and methionine biosysnthesis.

Scheme 33




The intriguing process of reduction of carbondioxide to methane in methanobacteria is also depending on a pterin derivative to convert the formyl into the methyl stage. The cofactor tetrahydromethanopterin (**56**) shows some structural relationship to tetrahydrofolate and does not unexpectedly reveal the same type of reactivity [49]. We have so far recently succeeded in synthesizing the left part of methanopterin (**55**) [50] and hope to be able in the near future to attach the ribosyl-phosphoryl-hydroxyglutarate moiety in the appropriate manner to get the native structure **55**.

Scheme 34

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czuk, E. Kleiner, W. R. Knappe, Y. Kobayashi, G. Konrad, W. Leskopf, E. Liedek, R. Lockart, R. Lohrmann, H. Lutz, S. Matsuura, R. Mengel, S. Murata, G. Nübel, A. Perez-Rubalcaba, D. D. Perrin, V. J. Ram, F. Reisser, W. J. Richter, H. Rokos, K. Rokos, M. Rukwied, H. Schlobach, D. Schmidt, H. J. Schneider, D. Söll, W. Southon, R. Soyka, T. Sugimoto, H. Steppan, N. Theobald, H. Traub, E. Uhlmann, M. Wiesenfeldt, H. Yamamoto, I. Ziegler, and H. Zondler.

## REFERENCES

[1] F. G. Hopkins, Proc. Chem. Soc., **5**, 117 (1889); F. G. Hopkins, *Ber.*, **24**, 724 (1889); F. G. Hopkins, Nature (London), **40**, 335 (1889).

[2] F. G. Hopkins, *Philos. Trans. R. Soc London, Ser.B*, **186**, 661 (1889).

[3] H. Wieland and C. Schöpf, *Ber.*, **58**, 2178 (1925).

[4] C. Schöpf and H. Wieland, *Ber.*, **59**, 2067 (1926).

[5] H. Wieland, H. Metzger, C. Schöpf and M. Bülow, *Liebigs Ann. Chem.*, **507**, 226 (1933).

[6] R. Purrmann, *Liebigs Ann. Chem.*, **544**, 182 (1940).

[7] R. Purrmann, *Liebigs Ann. Chem.*, **546**, 171798 (1940).

[8] R. Purrmann, *Liebigs Ann. Chem.*, **548**, 284 (1941).

[9] C. Schöpf, R. Reichert, and K. Riefstahl, *Liebigs Ann. Chem.*, **548**, 82 (1941).

[10] W. Pfleiderer, *Chem. Ber.* **95**, 2195 (1962).

[11] W. Pfleiderer, *Z. Naturforsch.*, **18b**, 420 (1963).

[12] W. Pfleiderer, in Physical Methods in Heterocyclic Chemistry, A. R. Katritzky, ed, Vol **1**, Academic Press New York, 1963, P 177.

[13] A. Albert, D. J. Brown, G. Cheeseman, *J. Chem. Soc.*, 1620 (1952).

[14] A. Albert in Advances in Heterocyclic Chemistry, A. R. Katritzky, ed, Vol **4**, Academic Press New York, 1965, p 1.

[15] A. Albert, *Angew. Chem.*, **79**, 913 (1967).

[16] D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 3443 (1956).

[17] D. Mohr, Dissertation University of Konstanz, 1991.

[18] W. Pfleiderer, J. W. Bunting, D. D. Perrin and G. Nübel, *Chem. Ber.*, **99**, 3503 (1966).

[19] W. Pfleiderer, J. W. Bunting, D. D. Perrin and G. Nübel, *Chem. Ber.*, **101**, 1072 (1968).

[20] W. Pfleiderer, R. Mengel and P. Hemmerich, *Chem. Ber.*, **104**, 2273 (1971).

[21] W. Pfleiderer, Angew. Chem., Int. Ed. Engl, **3**, 114 (1964).

[22] J. C. Nixon in Folates and Pterins, Vol **2**, R. L. Blakley and S. J. Benkovic, eds. John Wiley & Sons, New York, 1985, p 1.

[23] N. Theobald and W. Pfleiderer, *Chem. Ber.*, **111**, 3385 (1975).

[24] M. Böhme, W. Hutzenlaub, W. J. Richter, E. F. Elstner, G. Huttner, J. von Seyerl, and W. Pfleiderer, *Liebigs Ann. Chem.*, 1705 (1986).

[25] H. Rokos and W. Pfleiderer, *Chem. Ber.*, **104**, 739 (1971).

[26] R. Mengel and W. Pfleiderer, *Chem. Ber.*, **111**, 3790 (1978).

[27] W. Pfleiderer, M. Kappel and R. Baur, In Biochemical and Clinical Aspects of Pterdines, Vol. **3**; W. Pfleiderer, H. Wachter and H. Ch. Curtius, eds, W. de Gruyter & Co., Berlin, 1984, p 3.

[28] R. Gottlieb, W. Pfleiderer, *Chem. Ber.*, **111**, 1763 (1978).

[29] Y. Kobayashi, Y. Iitaka, R. Gottlieb and W. Pfleiderer, *Acta Crystallogr.*, **B33**, 2911 (1977).

[30] R. Baur, E. Kleiner and W. Pfleiderer, *Liebigs Ann. Chem.*, 1789 (1984); R. C. Boruah, R. Baur, W. Pfleiderer, *Croat. Chem. Acta*, **59**, 183 (1986).

[31] R. Huisgen, *Angew. Chem.*, **67**, 756 (1955).

[32] J. P. Dirlam, B. W. Cue and K. J. Gombatz, *J. Org. Chem.*, **43**, 76 (1978).

[33] C. Wentrup, C. Thetaz, E. Tagliaferri, H. J. Linder, B. Kitschke, H. W. Winter and H. P. Reisenauer, *Angew. Chem.*, **92**, 556 (1980).

[34] T. Watanabe, J. Nishiyama, R. Hirate, K. Uehara, M. Inoue, K. Matsumoto and A. Ohta, *J. Heterocyclic Chem.*, **20**, 1277 (1983).

[35] L. Giammanco and F. P. Invidata, *Heterocycles*, **23**, 1459 (1985).

[36] H. W. Moore, *Acc. Chem. Res.*, **12**, 127 (1979).

[37] W. Pfleiderer, *Heterocycles*, **28**, 203 (1989).

[38] E. P. Janulis and A. J. Arduengo III, *J. Am. Chem. Soc.*, **105**, 3563 (1983). E. P. Janulis, S. R. Wilson and A. J. Arduengo III, *Tetrahedron Letters*, **25**, 405 (1984).

[39] W. Pfleiderer in Comprehensive Heterocyclic Chemistry, Vol **3**, Part 2B, A. R. Katritzky and C. W. Rees, eds, Pergamon Press, Oxford, 1984, p 263.

[40] F. Weygand, A. Wacker and Schmied-Kowarzik, *Chem. Ber.*, **82**, 25 (1949).

[41] M. Viscontini, R. Provenzale, *Helv. Chim. Acta*, **51**, 1495 (1968); M. Viscontini, R. Provenzale, S. Ohlgart and J. Mallevialle, *Helv. Chim. Acta*, **53**, 1202 (1970).

[42] B. Schircks, J. H. Bieri and M. Viscontini, *Helv. Chim. Acta*, **60**, 211 (1977).

[43] M. Kappel, R. Mengel and W. Pfleiderer, *Liebigs Ann. Chem.*, 1815 (1984).

[44] R. Soyka, W. Pfleiderer and R. Prewo, *Helv. Chim. Acta*, **73**, 808 (1990).

[45] G. M. Brown in Folates and Pterin, Vol **2**, R. L. Blakley and S. J. Benkovic, eds, John Wiley & Sons, New York, 1985, p 115.

[46] T. G. Wilson and K. B. Jacobson, *Biochem. Genet.*, **15**, 307 (1977).

[47] K. B. Jacobson, D. Dorsett, W. Pfleiderer, J. A. McCloskey, S. K. Sethi, M. V. Buchanan and I. B. Rubin, *Biochemistry*, **21**, 5700 (1982).

[48] G. J. Wiederrecht, D. R. Paton and G. M. Brown, *J. Biol. Chem.*, **256**, 10399 (1981).

[49] G. D. Vogels, J. I. Keltjens and C. van der Drift in Biology of Anaerobic Microorganisms, A. J. B. Zehnder, eds, John Wiley & Sons, New York, **1988**, p 707.

[50] G. Heizmann, Ph.D. Thesis, University of Konstanz, 1991.