The status of blue-green bile pigments of butterflies, and their phototransformations

by M. Barbier[1]

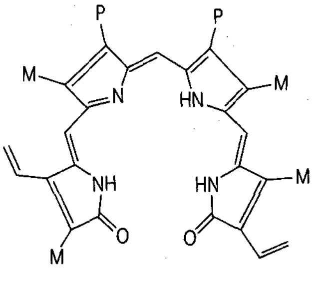
Institut de Chimie des Substances Naturelles, CNRS, F-91190 Gif sur Yvette (France)

Summary. Blue-green bile pigments are found in adults or larvae of many species of butterfly. They are reviewed here. In contrast to the presence of biliverdin IX α **1** in some insect species, many Lepidoptera contain pterobilin **2** (biliverdin IX γ), phorcabilin **3**, and sarpedobilin **4**, derived from **2** by cyclization. Pigments **3** and **4** are members of a new natural family of heterocyclic systems which can also be obtained *in vitro* from **2**, by irradiation under visible light. This review presents the information so far available about the chemistry and biochemistry of these pigments, together with a discussion about the possible biological significance of their photosensitivity.

Introduction

For a long time, the blue-green colors encountered among Lepidoptera have been thought to be due to the diffraction of light falling on specialized wing structures. This is true in many species, for example for the so-called 'metallic' blue colors found in the *Morpho* group, but it is not absolutly general. Accumulation of chlorophyll from the feeding sources has been believed to be responsible for the green color observed in the integuments of larvae. It was only in 1940 that Wieland and Tartter[2] succeeded in isolating the blue-green bilin from the wings of several *Pieris* species; this tetrapyrrolic pigment was named pterobilin. Later, in 1952, Hackman[3] demonstrated that pterobilin was also present as a circulating pigment in the hemolymph of larvae belonging to the *Pieris* family, where it occurs in the form of a high molecular weight chromoprotein. Biliverdin IXα **1** had been detected before among Orthoptera, Cheleutoptera, Dictyoptera, Odonata and Planipenes[4-11].

After a series of investigations on insect bilins belonging to the IXα series, as mentioned above, structural determinations were extended to the pterobilin group. In this review we present the results of chemical and biochemical research performed in our laboratory during the last 13 years. A discussion about hypotheses concerning the possible biological significance of this group of bile pigments in Lepidoptera is added.



Biliverdin IXα **1**

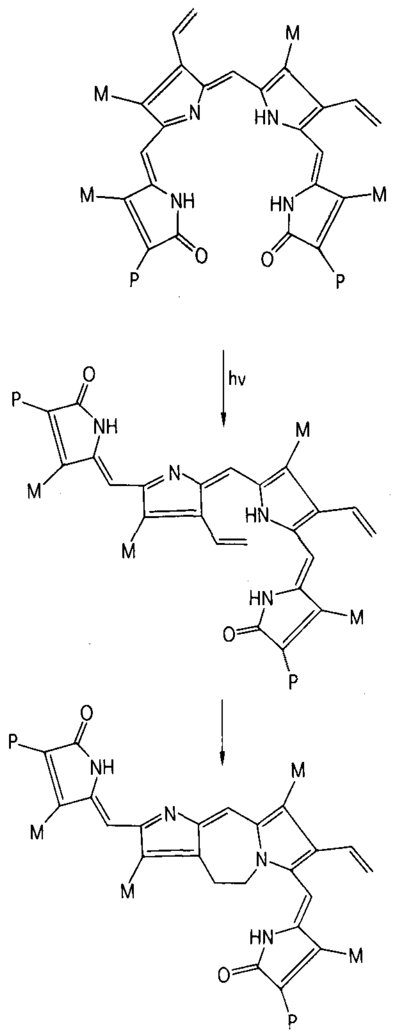
M = CH_3, P = CH_2-CH_2-COOH

The chemical structures of the blue-green bile pigments of Lepidoptera

The structure **2** was established in 1968 for pterobilin obtained from *Pieris brassicae*[12,13] relating this bilin to the IXγ series, and it was the first demonstration of a natural IXγ tetrapyrrole. A screening among Lepidoptera imagos and larvae[14] (27 species) showed pterobilin to be present not only in Pieridae but also in Papilionidae and Nymphalidae. During the course of this systematic search, 2 new blue-green bile pigments were isolated; the 1st, from *Papilio phorcas,* was named phorcabilin and the 2nd, from *Papilio sarpedon,* was named sarpedobilin[14,15]. Structures **3** and **4** were established for phorcabilin[16] and sarpedobilin[17] indicating that these bilins could be formed by some internal cyclizations of pterobilin. The 2 substances are the first members of a new family of natural heterocycles derived from a tetrapyrrolic pigment.

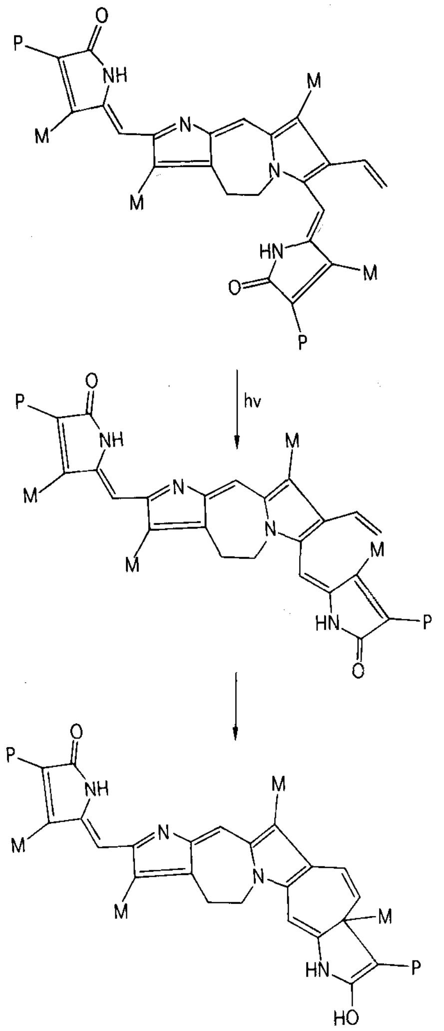
The screening was later extended to a total of 100 species[18] (10 families) and it underlined the wide distribution of pterobilin, the relative scarcity of phorcabilin and the rarity of sarpedobilin. This last pigment is found in all *Papilio sarpedon* subspecies investigated, in *Papilio weiskei* and as a minor component in some other butterflies. Phorcabilin is relatively abundant in Attacidae of the *Actias* group, *Argemma mittrei, Graellsia isabella* and *Antherea pernyi* (larvae), and also in trace amounts in some pterobilin-containing individuals. The statistics, on the basis of the Lepidopteran species examined so far[14,18,19] indicates that about 72% contain pterobilin, 15% phorcabilin and 5% sarpedobilin. In Noctuidae and Geometridae, the green bile pigment often detected in larvae or adults is apparently of a different type, which is quite unstable during chemical treatment. At least in the case of *Thaumetopoea pityocampa*[20] it could be related to the usual IXα family. Among the Sphingidae, *Euchloron megaera*[14,19] has a deep emerald green color, due to an unidentified (hot) water soluble pigment which is not a tetrapyrrole and is possibly derived from a plant food polyphenol.

Pterobilin 2



Phorcabilin 3

Phorcabilin 3



Sarpedobilin 4

$M = CH_3$, $P = CH_2\text{-}CH_2\text{-}COOH$

Photo-isomerizations and photo-cyclizations in the IXγ series of butterflies bile pigments: from pterobilin to phorcabilin (left) and from phorcabilin to sarpedobilin (right).

Biosynthesis of Lepidopteran bile pigments

The biosynthesis of pterobilin 2 from lebelled glycine has been demonstrated in *Pieris brassicae*[13]. By various experiments using labelled precursors[18,21,22] the following scheme has finally been established: acetate → glycin → δ-aminolevulinic acid → corpro-porphyrinogen-III → protoporphyrin IX → pterobilin → phorcabilin → sarpedobilin. This scheme is identical, up to protoporphyrin IX, with the biosynthetic pathway generally found among vertebrates. The main differences are noticed from this point, in connection with the specific oxidative cleavage of the porphyrin ring in the γ position in butterflies, instead of the usual α position. The mechanism of the in vivo transformation of pterobilin into phorcabilin and sarpedobilin is still unknown. In spite of the easy photo-conversion (see under) of pigment 2 into 3 and 4 by visible light in vitro, such a mechanism has not been demonstrated in vivo; what is more, the chromoprotein of pterobilin from *Pieris brassicae* is photoresistant to cyclization of the bilin and the intervention of an enzyme cannot be excluded. Another possibility would be a phototransformation occurring in the free form of the bile pigment at the moment of larval moulting, as the biosynthesis has been shown to be more active at this time.

Phototransformations of the IXγ bile pigments

A reaction has been discovered[18,23,24] showing the in vitro phototransformation of pterobilin 2 into phorcabilin 3 and of the latter into sarpedobilin 4 under the action of visible light. The special features of the isomerization-cyclization reaction in the IXγ series[24] are connected with the possibility of the addition of the 2 vinyl groups in the central pyrrole rings, to the neighbour pyrrolic nitrogen atoms. The same reaction is observed with the non-natural IXδ bilin[25] where only 1 of the 2 vinyl substituents in a good position cyclizes under similar conditions. The following scheme summarizes the situation for the in vitro phototransformations of pigment **2** (pterobilin):

$$\underset{\text{(pterobilin)}}{\mathbf{2}} \xrightarrow[\text{1st cyclization}]{h\nu} \underset{\text{(phorcabilin)}}{\mathbf{3}} \xrightarrow[\text{2nd cyclization}]{h\nu} \underset{\text{(sarpedobilin)}}{\mathbf{4}}$$

Concluding remarks and hypotheses concerning the biological significance of butterfly IXγ pigments

The bile pigments of butterflies represent the only known occurrence in nature of bilins belonging to the IXγ series. The 'raison d'être' of this particular metabolic feature is unknown and the phototransformations observed in vitro have not yet been established in vivo.

A role in the larval development of *Pieris brassicae* has been proposed[26-28] and represents a valuable hypothesis. In this scheme, pterobilin would act as a photoreceptor for the red wave-lengths of the visible spectrum. This would correspond to a biological clock connected with the day-night relationships and hence with the determination of diapause in larvae. Such a mechanism could be bound to the 1st step of the phototransformation; that is, the photo-isomerization of pterobilin at the methen bridges, as found with the plant phytochrome. A molecular basis for metering time through pigments present in membranes has been emphazised[31]; the whole mechanism being related to the existence of 'chronogen segments'. Vuillaume et al.[32-34] have shown that the photoreceptor role of pterobilin could be connected in *Pieris brassicae* integuments with the production of ATP.

Similar questions concerning the biological significance of such bile pigments when deposited in Lepidopteran wing membranes have been discussed recently[29]. They certainly play a role in crypsis[30] both in caterpillars and butterflies, as the most frequent background in nature is of course due to the chlorophyll in leaves. In butterflies, there is evidence for morphological modifications of the scales[29] which ensure a better exposure to light in pterobilin-containing species, suggesting that the pigment fulfils a photoreceptive function. A hypothesis has been produced which suggests that this could be connected with a system of heat transference within the butterfly wing.

Added in proof: The iron complex of a synthetic γ-hydroxy derivative of protoporphyrin IX undergoes oxidative ring opening to give pterobilin and this provides a model for the study of its biosynthesis. (A.H. Jackson, R.M. Jenkins, D.M. Jones and S.A. Matlin, J. chem. Soc., chem. Commun. *1981*, 763).

1 Acknowledgments. Thanks are due to Prof. D.H.R. Barton, E. Lederer and J. Bergerard, for their interest and for discussions.
2 H. Wieland and A. Tartter, Ann. Chem. *545*, 197 (1940).
3 R.H. Hackman, Archs Biochem. Physiol. *41*, 166 (1952).
4 M. Passama-Vuillaume, C.r. Acad. Sci. Paris *258*, 6549 (1964).
5 M. Passama-Vuillaume and B. Levita, C.r. Acad. Sci. Paris *263*, 1001 (1966).
6 M. Passama-Vuillaume, Bull. Soc. zool. Fr. *90*, 485 (1965).
7 M. Vuillaume and M. Grouselle, Bull. Soc. Chim. biol. *50*, 681 (1968).
8 M. Vuillaume, Bull. Biol. *2*, 147 (1968).
9 W. Rüdiger, W. Klose, M. Vuillaume and M. Barbier, Bull. Soc. Chim. biol. *51*, 559 (1969).
10 W. Rüdiger and W. Klose, Experientia *26*, 498 (1970).
11 M. Passama-Vuillaume and M. Barbier, C.r. Acad. Sci. Paris *263*, 924 (1966).
12 W. Rüdiger, W. Klose, M. Vuillaume and M. Barbier, Experientia *24*, 1000 (1968).
13 W. Rüdiger, W. Klose, M. Vuillaume and M. Barbier, Experientia *25*, 487 (1969).
14 M. Vuillaume and M. Barbier, C.r. Acad. Sci., Paris *268*, 2286 (1969).
15 M. Choussy, M. Barbier, W. Rüdiger and W. Klose, Comp. Biochem. Physiol. *44B*, 47 (1973).
16 M. Choussy and M. Barbier, Helv. chim. Acta *58*, 2651 (1975).
17 M. Bois-Choussy and M. Barbier, Experientia *33*, 1407 (1977).
18 M. Bois-Choussy, Thesis, Orsay University, Paris 1977.
19 M. Vuillaume, M. Choussy and M. Barbier, Bull. Soc. zool. Fr. *95*, 19 (1970).
20 M. Choussy, J. Larrouquère, M. Barbier and M. Lamy, C.r. Acad. Sci. Paris *277* Ser. D. 365 (1973).
21 M. Choussy, M. Barbier and M. Vuillaume, Biochimie *57*, 369 (1975).
22 M. Bois-Choussy, T. Hidaka and M. Barbier, unpublished result.
23 M. Choussy and M. Barbier, C.r. Acad. Sci. Paris *282* Ser. C, 619 (1976).
24 M. Bois-Choussy and M. Barbier, Heterocycles *9*, 677 (1978).
25 M. Bois-Choussy and M. Barbier, Helv. chim. Acta *63*, 1098 (1980).
26 M. Barbier, J. Bergerard, B. Hurpin and M. Vuillaume, C.r. Acad. Sci. Paris *271*, 342 (1970).
27 M. Vuillaume, J. Seugé and J. Bergerard, Int. J. Chronobiol. *2*, 181 (1974).
28 M. Vuillaume and J. Bergerard, Chronobiologia *5*, 286 (1978).
29 A.C. Allyn, M. Barbier, M. Bois-Choussy and M. Rothschild, Antenna, Lond. 29 (1981).
30 M. Barbier, Alexanor *7*, 312 (1972).
31 L.N. Edmunds and K.J. Adams, Science *211*, 1002 (1981).
32 M. Vuillaume, R. Calvayrac and M. Best-Belpomme, Biol. cell. *35*, 71 (1979).
33 M. Vuillaume and M. Best-Belpomme, Experientia *36*, 1169 (1980).
34 M. Vuillaume and Y. Dattée, Archs Zool. exp. gén. *121*, 159 (1980).