Vol. 110, No. 3, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 10, 1983 Pages 779-782

BIOSYNTHESIS OF THE BILE PIGMENT SARPEDOBILIN FROM [^{14}C]PTEROBILIN BY PAPILIO SARPEDON (LEPIDOPTERA)

Michèle Bois-Choussy and Michel Barbier

Institut de Chimie des Substances Naturelles, CNRS,
91190 Gif sur Yvette, France

Received December 17, 1982

Sarpedobilin 3, the main blue-green tetrapyrrolic bile pigment of the butterfly Papilio sarpedon, is present at the different larval instars and in the adults. Using labelled pterobilin 1, it is established that this compound is the precursor of sarpedobilin in larvae of the 4th instar.

A systematic search for blue-green bile pigments in insects (1-11) has been carried out in this laboratory for several years. Three substances have been identified from butterflies, pterobilin 1, the mono cyclized phorcabilin 2 and the dicyclised sarpedobilin 3 (3-8). Pterobilin is widely represented among species, imagos and larvae, while phorcabilin and sarpedobilin are more restricted. Sarpedobilin is present in all Papilio sarpedon subspecies investigated, also in P. weiskei and in P. phorcas in trace amount.

The biosynthesis of pterobilin from labelled glycin has been demonstrated in Pieris brassicae and using various precursors (9) a general scheme was finally proposed: acetate---> glycin---> δ-aminolevulinic acid---> coproporphyrinogen-III---> protoporphyrin-IX---> pterobilin. This scheme is identical up to protoporphyrin-IX with the biosynthetic pathway generally found in vertebrates, but the mechanism of the oxidative cleavage at the δ position as well as the mechanism of the cyclizations of pterobilin 1 into 2 and 3 *in vivo* are still to be demonstrated. The *in vitro* phototransformation of 1 into 2 and 3 has been observed also the thermal rearrangement of pterobilin 1 into phorcabilin 2 (11) but the *in vivo* rearrangement of 1 through irradiation is not established.

0006-291X/83/030779-04$01.50/0

The case of the linear tetrapyrrolic pigments of butterflies is unique in nature and the "raisons d'être" of such pecularities are still limited to hypotheses and waiting for further experiments.

In the present publication, we report the determination of sarpedobilin in all instars and in the imagos of Papilio sarpedon and the transformation of ^{14}C-labelled pterobilin into sarpedobilin by larvae of the 4th instar.

MATERIAL AND METHODS

14C-pterobilin was prepared through injection of 4-^{14}C-δ-aminolevulinic acid (0.2 mCi, 47 mCi/mM) in 200 larvae of Pieris brassicae at the 4th instar and it has been further purified by SiO_2 thin layer chromatography (tlc) of the dimethyl ester, obtained after treatment with methanol-sulfuric acid 10%. After hydrolysis of the diester (HCl, 10 min., 100°) 0.09 mg of pterobilin, spectrophotometrically determined at 650nm in methanol (ε 13500) is obtained (1.47×10^{-4} mM, specific radioactivity 1.5×10^{10} dpm/mM). The technical details concerning the extractions of the pigments, chromatographical conditions, identifications by mass spectrometry or nmr, have been fully reported (4). The spectrophotometrical determinations have been performed on an automatic scanner Leres S 66 and radioactivity measured by the usual scintillation technic in toluene, quenching corrected, with an apparatus Intertechnique SL 30.

3×10^6 dpm of labelled pterobilin were injected in DMF to 14 larvae of P.sarpedon at the 4th instar and sarpedobilin extracted after the 5th day, following reported procedures (4,5) as sarpedobilin dimethyl ester. After SiO_2 tlc purification until constant radioactivity, the pigment was spectrophotometrically determined at 600nm in methanol (ε 60.000) leading to 5×10^{-5} mM of total radioactivity 3×10^3 dpm, yield of the incorporation 0.1%.

RESULTS

Sarpedobilin has been spectrophotometrically determined in the wings of P.sarpedon imagos and in the larva from the 1st to the 5th instar, and the results obtained are reported in the table. It appears that the most active phases of the biosynthesis of this bile pigment occur at the 4th and 5th instars and during pupation. Consequently, we have choosen the 4th instar to demonstrate the biosynthesis of sarpedobilin from pterobilin in the following experiment.

^{14}C-pterobilin was injected to larvae at the 4th instar and sarpedobilin extracted after the 5th day as the dimethyl ester, following the already reported procedure (4). After SiO_2 thin layer chromatography until constant radioactivity, the pigment was spectrophotometrically determined, leading to 5×10^{-5} mM of sarpedobilin dimethyl ester of total

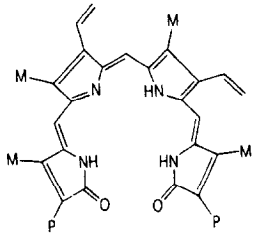
Table. Spectrophotometrical determination of sarpedobilin 3 in P. sarpedon adults and larvae (using ε 60.000 at 600nm in methanol for the dimethyl ester).

	Larvae					Adults
	L1	L2	L3	L4	L5	(wings)
dry weight g/individual	0.001	0.002	0.011	0.026	0,177	0.07
Sarpedobilin µg/individual	0.01	0.04	0.27	1.10	3.30	6.30
Sarpedobilin µg/g dry weight	8.5	16.5	25	42	19	90

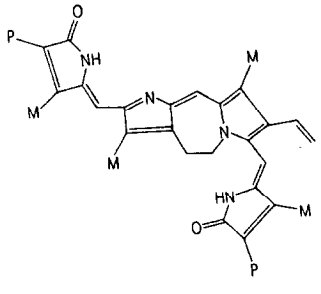
radioactivity $3x10^3$ dpm, representing a yield of incorporation, calculated after the purifications, of 0.1%.

This experiment establishes that pterobilin 1 is the precursor of sarpedobilin 3 through a double cyclisation of the central vinyl groups on the neighbour nitrogen atoms *in vivo*; however, the mechanism of this transformation remains to be demonstrated.

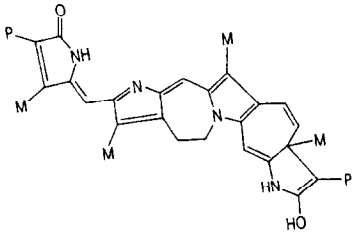
In *Pieris brassicae* (12-14) pterobilin has been presumed to have a role in photoreception in connection with a sort of biological clock for diapause time metering at the different instars. In adult butter-



1



2



3

1: Pterobilin; 2: phorcabilin; 3: sarpedobilin; M=CH_3; P=CH_2CH_2COOH or $CH_2CH_2COOCH_3$ in the methyl esters.

flies,it has been supposed to have a role in heat transference (15) as wing scales are often morphologically modified where pterobilin accumulates,as to facilitate photoreception.However,the biological significance of the transformation of pterobilin into phorcabilin and sarpedobilin is not yet known,nor the real support of such transformations (free state of the pigments or chromoproteins).Other observations will be necessary to establish the details of such transformations in Lepidopters .

Acknowledgements.Thanks are due to Professor M.T.Hidaka for the breeding of P.sarpedon and to Professor J.Bergerard for his interest and discussions.

REFERENCES

1.Rüdiger,W.,Klose,W.,Vuillaume,M.,and Barbier,M.(1969).Bull.Soc.Chim. Biol.,51,559-562.
2.Vuillaume,M.and Barbier M.(1966).Compte - rendus Acad.Sci.Paris,263, 924-925.
3.Vuillaume M.,and Barbier M.(1969).Compte - rendus Acad.Sci.Paris, 268,2286-2289.
4.Choussy,M.,and Barbier,M.(1975).Helv.Chim.Acta,58,2651-2661.
5.Bois-Choussy,M.,and Barbier,M.(1977).Experientia,33,1407-1410.
6.Bois-Choussy,M.,(1977) Thesis,Orsay University,1-122.
7.Vuillaune,M.,Choussy,M.,and Barbier,M.(1970).Bull.Soc.Zool.Fr.,95, 19-28.
8.Barbier,M.(1981).Experientia,37,1060-1062.
9.Rüdiger,W.,Klose,W.,Vuillaume,M.,and Barbier,M.(1969).Experientia,25, 487-488.
10.Choussy,M.,Barbier,M.,and Vuillaume,M.(1975).Biochimie 57,369-373.
11.Bois-Choussy,M.,and Barbier,M.(1978).Heterocycles 9,677-690.
12.Barbier,M.,Bergerard,J.,Hurpin,B.,and Vuillaume,M.(1970).Compte-rendus Acad.Sci.Paris,271,342-345.
13.Vuillaume,M.,and Bergerard,J.(1978).Chronobiologia,5,286-292.
14.Vuillaume,M.,and Dattée,Y.(1980).Arch.Zool.Exper.Gen.,121,159-164.
15.Allyn,A.C.,Barbier,M.,Bois-Choussy,M.,and Rothschild,M.(1981).Antenna, Londres,29-31.