On the Structure of Pterobilin, the Blue Pigment of *Pieris brassicae*

From the wings of the common cabage butterfly, *Pieris brassicae*, and of other Lepidoptera, WIELAND and TARTTER[1] isolated a blue bilin which behaved similarly to mesobiliverdin-IXα (glaucobilin) (1a) in stability to concentrated sulphuric acid and in spectral properties, but proved to be different from this pigment and from biliverdin-IXα (1b) in the Debye-Scherrer-diagram. The pigment was called pterobilin and was thought to be isomeric to 1b. HACKMAN[2] concluded from the spectral properties of the hemolymph bilin of caterpillars of *P. brassicae* and other Lepidoptera that this pigment is identical with pterobilin and, supporting an earlier assumption[3], probably with 1a. The tegumental bilin of green pupae of *P. rapae crucivora* has been claimed to be identical with the pigment of the hemolymph[4] and, probably, with 1a or 1b. We want to present evidence here that the tegumental bile pigment of caterpillars of *P. brassicae* is biliverdin-IXγ (2a), an isomer of 1b.

The chromoproteid of 200 caterpillars[5] has been prepared[6] by excising the animals dorsally, removing the organs, washing and then grinding the integuments with the minimum amount of water, and filtering immediately. The raw chromoproteid is precipitated from the greenish filtrate with solid $(NH_4)_2SO_4$ (100% saturation) and collected by centrifugation – after removal of dark brown material at slow speed – at 18,000 rpm; after repitition of the $(NH_4)_2SO_4$ treatment it is lyophilized and stored under vacuum at 0 °C.

The pigments are extracted from the chromoproteid with methanol/10% H_2SO_4 at 8 °C and, after esterification, transferred to chloroform. After thin-layer chromatography (TLC: silica gel G, benzene/petrol/methanol = 60:5:3), the coloured zones are eluted with acetone giving the methyl pheophorbides (a) (4.7 mg) and (b) (0.66 mg) and pterobilin dimethylester (0.09 mg)[7].

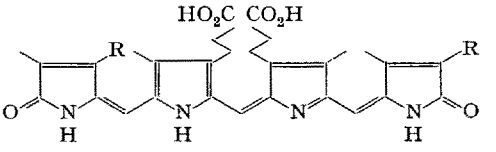
Pterobilin dimethylester yields the imides 3 and 4 on chromic acid oxidation (1% CrO_3 in 2n H_2SO_4)[8], thus proving the presence of vinyl side chains and excluding ethyl side chains (to be expected if it were a meso type bilin). On chromate oxidation (1% $K_2Cr_2O_7$/1% $KHSO_4$)[8], it yields the pyrrole dialdehyde 5. This means that the vinyl groupings both must be located at the inner rings, because pyrrole dialdehydes cannot be formed from oxygen substituted outer rings of bilins.

If pterobilin is derived from protoporphyrin-IX, the only naturally occurring isomer of the protoporphyrin series, it must be biliverdin-IXγ (2a). The identity is proved by comparison of the esterified pigment with authentic 2b isolated from the mixture of isomeric biliverdins obtained by coupled oxidation of pyridine protohaemochrome-IX and ascorbic acid[9]. The pigments of both sources are inseparable from each other in TLC – e.g. Rf 0.36 in benzene/dioxane/acetic acid = 12:2:1 – but are separated from the dimethylester of biliverdin-IXα (Rf 0.22). The absorption spectra (Table) further support the identity of pterobilin and biliverdin-IXγ; the spectrum of biliverdin-IXα is slightly but significantly different.

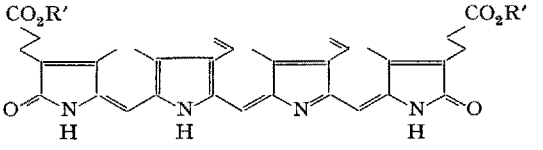
Absorption maxima (nm) of biliverdin dimethylesters

Pigment	Methanol/5% NH_3	Methanol/5% HCl	Methanol/5% $Zn(OAc)_2$
Pterobilin	266; 372; 660	260; – 357; 700	266; 381; 700
Biliverdin-IXγ	266; 372; 657	261; – 360; 700	266; 382; 705
Biliverdin-IXα	261; 376; 653	261; 308; 376; 700	261; 390; 715

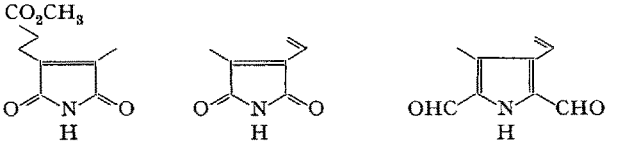
Further experiments will show whether pterobilin is formed by oxidative ring fission of a protoporphyrin-IX compound at the γ-methine bridge or whether it arises in an independent way.



1a: R = C_2H_5
1b: R = $CH{=}CH_2$



2a: R′ = H
2b: R′ = CH_3



3 4 5

Zusammenfassung. Aus dem Tegument von Raupen des Kohlweisslings (*Pieris brassicae*) wird das bereits früher beschriebene Pterobilin isoliert, das auf Grund seiner Abbauprodukte und der chromatographischen und spektralen Eigenschaften als Biliverdin-IXγ identifiziert wird.

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