Presence and Biosynthetic Implications of β,β-Carotene-2-one in the Moth *Cerura vinula*

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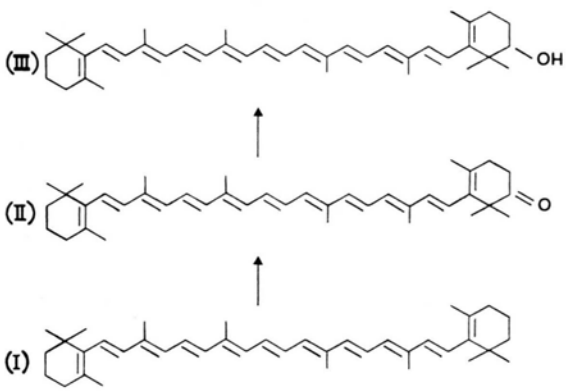
Z. Naturforsch. **34 c**, 483–484 (1979);
received January 29, 1979

Carotenoids, β,β-Carotene-2one, Metabolism, Insects, *Cerura*

β,β-Carotene-2-one was isolated in minute amounts from *Cerura vinula* and identified on the basis of spectra, hydride reduction, and chromatography. The implications on the biosynthesis of 2-hydroxylated carotenoids in insects are discussed.

Since the first discovery of 2-hydroxylated carotenoids in a green alga [1] pigments of this substitution type have recently been isolated from several insects such as the moth *Cerura vinula* [2] and stick insects of the genera *Carausius* [3–5], *Ectatosoma* [5, 6], and *Acrophylla* [6]. From these stick insects a series of novel carotenoids with 2-one and 3,4-didehydro-2-one structure, respectively, have been isolated in addition [4–6], and the hypothesis was offered that the hydroxy compounds are derived from the corresponding ketones and not synthesized directly. In *Cerura*, however, only β,β-carotene-



2-ol (**III**), which is present in large amounts, has been identified up to now [2]; so, the question arose, whether in this insect the 2-hydroxy group could be introduced directly [7]. A similar mechanism has been suggested for the biosynthesis of such pigments in the green alga [1], however, this substitutions is thought to be tightly coupled to the ring closure, an enzymatic step not operating in animals generally [8]. In a direct search for the missing β,β-carotene-2-one (**II**) in *Cerura* we now succeeded in the isolation of low amounts of this compound, thus demonstrating that similar biosynthetic mechanisms may exist in this moth and in stick insects.

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0341-0382 / 79 / 0500-0483 $ 01.00/0

Materials and Methods

About sixty pupae were extracted with acetone and methanol and saponified with 5% KOH in methanol [2, 9]. A large amount of unsaponified material was removed by precipitation from petroleum ether (50–70 °C) at −30 °C. The carotenoids were separated by preparative partition TLC on silica gel-G (Merck) developed with petroleum ether (100–140 °C)/propanol-2 (60:3).

The faint yellow zone moving between the carotene zone and the mono-hydroxy carotenoid was collected and repurified by multiple development with the same solvent made less polar (60:2). The carotenoid migrated together with lipidic material, which could not be removed by either change of the solvent or storage in the cold. The piment zone was eluted with ethanol and reduced by addition of solid $NaBH_4$ at room temperature for 30 min. After a transfer to petroleum ether the reduction products were studied on silica gel-G again and on the adsorption layer ($CaCO_3$/MgO/$Ca(OH)_2$) [2] and compared with authentic samples. Electronic spectra were taken in acetone or ethanol with a Zeiss recording spectrophotometer type DMR 21.

Results

The carotenoid in question migrated roughly midway between β,β-carotene and β,β-carotene-2-ol (*cf.* ref. [2]) on silica gel-G indicating an intermediate polarity, which is in agreement with a carbonyl function. It co-chromatographed with β,β-carotene-2-one isolated from *Ectatosoma* and confirmed by mass spectrometry [5, 6]. The visible absorption spectrum, though disturbed by lipid material, exhibited the chromophore of β,β-carotene; consequently, the carbonyl group should not be conjugated to the polyene chain. When the carotenoid was treated with $NaBH_4$ in ethanol and then chromatographed on silica gel-G, its polarity was increased (*i. e.* its R_f-value was depressed) to that of

β,β-carotene-2-ol from both *Cerura* and the stick insects. The lipid zone, which co-migrated with the pigment before reduction, was unchanged and well separated, consequently. Nevertheless, the spectrum of the reduction product was impure due to the presence of other chromophores. After purification on the adsorption layer a typical β,β-carotene type spectrum was obtained with maxima at 452 and 478 nm in acetone; so, no significant spectral change occured during reduction. On adsorption TLC the product again co-chromatographed with authentic β,β-carotene-2-ol. The combination of these two TLC systems has been shown to distinguish very selectively between the different isomeric mono-ols [2]. Conclusively, the evidence obtained from chromatography, spectra and reduction is sufficient to confirm the presence of β,β-carotene-2-one (**II**) in *Cerura*. Roughly estimated, it amounts to approx. 1 μg or less per pupa; due to this trace quantity it had been overlooked in a preceding study [2].

Discussion

Due to the very low abundance of the 2-one (**II**) in *Cerura* one can hardly imagine, that this compound could be produced by oxidation of the 2-ol (**III**); in this case it should be an accumulating end product. In analogy to the carotenoid pathway suggested for stick insects [4 – 6] it is assumed (Fig.) that in *Cerura*, too, the keto compound (**II**) is the first stable metabolite of β,β-carotene (**I**) and is rapidly reduced to the 2-ol (**III**), which in fact accumulates during larval life up to 40% of total carotenoids [10]. If this view is correct, it is interesting to see the presence in very different orders of insects of carotenoid pathways producing metabolites of the same unusual type in the same way. Perhaps, this is related to the identical substitution sites in β,β-carotene (**I**) as precursor, affording identical molecular mechanisms of enzymatic attack different to those operating in plants obviously [1]. Other structural data stressing the metabolic analogy in *Cerura* and stick insects will be reported in a separate paper.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 87).

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