Presence and Biosynthetic Implications of \( \beta,\beta \)-Carotene-2-one in the Moth Cerura vinula

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\( \beta,\beta \)-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 Aerophylla [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 \( \beta,\beta \)-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 \( \beta,\beta \)-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.

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)/propan-2ol (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 \( \beta,\beta \)-carotene and \( \beta,\beta \)-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 \( \beta,\beta \)-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 \( \beta,\beta \)-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
**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.

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