Metabolites of β,β-Carotene in the Stick Insect, *Carausius morosus* Br.: Compounds with 2-One and 3,4-Didehydro-2-one Structure

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(Z. Naturforsch. **32 c**, 327–336 [1977]; received January 1/March 25, 1977)

Carotenoids, 2-Keto β,β-Carotenes, Insects, Metabolism

Six novel keto carotenoids have been isolated from the stick insect. The structure of the predominant red pigment has been assigned to 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione on the basis of electronic, infrared, and mass spectra. Minor pigments are 3,4-didehydro-β,β-carotene-2,2′-dione, 2′-hydroxy-3,4-didehydro-β,β-caroten-2-one, and 2′-hydroxy-β,β-caroten-2-one. Two other compounds, β,β-caroten-2-one and β,β-carotene-2,2′-dione, have been tentatively identified. The additional occurrence of β,β-caroten-2-ol and β,β-carotene-2,2′-diol has been reported recently. All hydroxylated pigments are mainly present as fatty-acid esters. During hydride reduction of the 3,4-didehydro-2-one compounds hydrogenation of the 3,4-double bond was observed as a side reaction.

A hypothetical scheme of carotenoid metabolism in the stick insect is proposed. According to this pathway the keto groups are introduced by a pseudo one-step reaction; the hydroxylated pigments are thought to be reduction products of the corresponding ketones. The proposed pathway is fitting more to physiological data than an alternative one based on the hydroxyl → carbonyl sequence.

Introduction

Metabolic transformation of carotenoids seems not to be a common capability of insects. Biogenesis of new carotenoids from dietary precursors has so far been postulated to occur among Coleoptera [1, 2], Lepidoptera [3], and Orthoptera [4]. Typically, the ketonic pigments echinenone, canthaxanthin, and astaxanthin have been identified as metabolic products which are formally derived from β-carotene by oxidation at C-4 or C-3,4 of the end rings; other products are analogous derivatives of lutein.

Willig [5] studying the stick insect, *Carausius morosus*, found a red keto carotenoid, which was not identical with a number of known pigments of this type, and which was therefore concluded to be a novel compound. He also reported the presence of predominant amounts of isocryptoxanthin (β,β-caroten-4-ol) and isozeaxanthin (β,β-carotene-4,4′-diol) in this insect. However, a reexamination, which was stimulated by results on the structure of the novel red pigment, presented evidence that the hydroxy compounds were not the 4-isomers mentioned, but β,β-caroten-2-ol and β,β-carotene-2,2′-diol [6].

The present paper is focusing on the structure of the major red pigment and other novel compounds, less in quantity, which were discovered during the large-scale isolation of carotenoids from *Carausius*. A general structural feature of these metabolites is a keto group at C-2, which in the red compounds is conjugated to the polyene chain via an additional 3,4-double bond. Carotenoids of this type have been neither found in nature nor obtained synthetically before. A brief report on the results has been presented recently [7].

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Materials and Methods

Insects

Carausius morosus Br. (Phasmidae, Orthopteroidea) was bred in the laboratory on leaves of *Hedera helix* at 18 °C under a light/dark cycle of 12:12 h. Adult and juvenile instars were used for carotenoid isolation. The viscera were removed prior to lyophilization.

Chromatography of carotenoids

The pigments were extracted and purified by thin-layer chromatography (TLC) as reported in preceding papers [8–10]. This procedure included partition TLC on silica gel-G and adsorption TLC on a mixture of $CaCO_3$, MgO, and $Ca(OH)_2$. Mixtures of petroleum ether (100 – 140 °C) and propanol-2 were used as solvents in both systems; for adsorption TLC also petroleum ether, acetone, and chloroform (130 : 25 : 25; v/v/v) were used. For analyt-

ical purposes precoated layers of polyamide (G 1600; Schleicher & Schüll, Dassel, Germany) were used in addition with petroleum ether (100–140 °C) and methyl ethyl ketone (50 : 3; v/v) as solvent. Samples for mass spectrometry were finally purified on silica gel 60 HR developed with n-hexane and acetone (120 : 12; v/v) [8].

Chemical reactions

Experimental details on saponification, acetylation, acid treatment and hydride reduction have been presented elsewhere [8–10].

Spectroscopy

Electronic spectra were recorded on a Zeiss DMR21 spectrometer. For calculation of pigment quantities a specific extinction coefficient of $E^{1\%}_{1cm} = 2200$ was used for all compounds [11]. – Infrared spectra were obtained on a Perkin-Elmer 325 spectrometer using KBr discs [10]. Mass spectrometry was performed at 70 and 12 eV on a Varian MAT CH5 instrument or an AEI MS902S spectrometer using the direct inlet systems. Perfluorokerosene was used as reference.

Reference carotenoids

β,β-Carotene-2,2′-diol and β,β-caroten-2-ol were isolated from the extracts of stick insects [6]. – Rhodoxanthin (4′,5′-didehydro-4,5′-*retro*-β,β-carotene-3,3′-dione) was obtained from the red fruits of *Taxus baccata* [12]. – Canthaxanthin (β,β-carotene-4,4′-dione) was a synthetic product of Hoffmann-La Roche, Basel. – 3,4,3′,4′-Tetradehydro-β,β-carotene was synthesized by N-bromosuccinimide (NBS) treatment of synthetic β,β-carotene (Merck) using 3 mol of NBS per mol carotene [13]. The purified product showed a symmetrical visible absorption maximum at 472 nm in acetone, which is in close agreement with published data (471 nm in hexane resp. light petrol [13–15]). – 3′,4′-Didehydro-β,β-caroten-4-one (λ_{max} = 470 nm in acetone), which had been produced by the NBS reaction, was reduced with $NaBH_4$ to obtain the corresponding 4-ol, which exhibited an asymmetrical absorption peak at 461 nm in acetone. This is coincident with reported absorption maxima of this chromophore (461-2 nm in hexane or light petrol [13–15]).

Results

On silica gel-G the carotenoid extract of *Carausius* was separated into twelve pigment zones before saponification and into eight fractions after this treatment, indicating the natural occurrence of several esterified carotenoids (Fig. 1). Two trace pigments were present in addition. The isolated

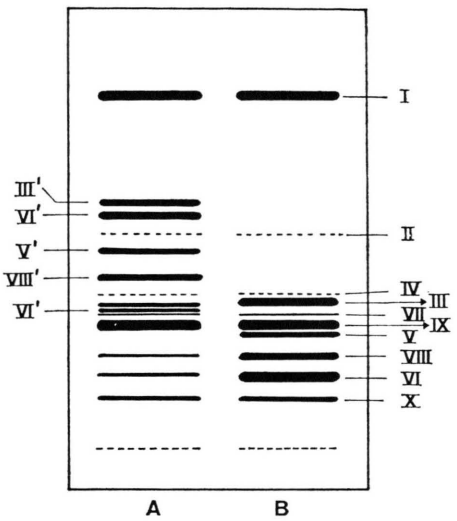

Fig. 1. Silica gel-G chromatogram of the carotenoids of *C. morosus*. A – native extract, B – saponified extract. **(I)** β,β-Carotene, **(II)** ββ,-Caroten-2-one, **(III)** β,β-Caroten-2-ol, **(IV)** β,β-Carotene-2,2′-dione, **(V)** 2′-Hydroxy-β,β-caroten-2-one, **(VI)** β,β-Carotene-2,2′-diol, **(VII)** 3,4-Didehydro-β,β-carotene-2,2′-dione, **(VIII)** 2′-Hydroxy-3,4-didehydro-β,β-caroten-2-one, **(IX)** 3,4,3′,4′-Tetradehydro-β,β-carotene-2,2′-dione, **(X)** β,ε-Carotene-3,3′-diol (lutein). Primed numbers in A refer to esters of the corresponding carotenoids. For structural formulae see Fig. 9.

ester fractions were saponified separately to get evidence on the identity of the esterified carotenoids as indicated in Fig. 1. Two pigments, β,β-carotene (β-carotene; hRf = 80) and β,ε-carotene-3,3′-diol (lutein; hRf = 11), have already been identified by Willig [5]; two others, formerly believed to be β,β-caroten-4-ol (isocryptoxanthin) and β,β-carotene-4,4′-diol (isozeaxanthin) by the same author, have now shown to be the corresponding 2-ol and 2,2′-diol, respectively [6]. Only a low quantity of the diol was referring to the free pigment (hRf = 17), most of it was present as mono-esters (hRf = 31) and, mainly, as diesters (hRf = 53) of fatty acids. Also the 2-ol was predominantly in esterified state (hRf = 56; free pigment hRf = 33). All other carotenoids were novel compounds with keto groups at position(s) C-2(2′) of the β-end rings.

3,4,3′,4′-Tetradehydro-β,β-carotene-2,2′-dione **(IX)**

This was the main red fraction of the *Carausius* carotenoids (hRf = 28). It did not change on sa-

ponification. On silica gel-G this pigment was slightly more polar than canthaxanthin (β,β-carotene-4,4′-dione); this difference was more pronounced on the adsorption plate (hRf = 23 and 31, respectively). On polyamide, however, the *Carausius* pigment was less adsorbed (hRf = 59) than canthaxanthin (hRf = 56); rhodoxanthin as a *retro* carotenoid did not migrate on polyamide [16] and was also most strongly adsorbed on the adsorption plate (hRf < 5). Therefore, the *Carausius* pigment should not have a *retro* structure.

The electronic spectrum (Fig. 2) exhibited a single symmetrical maximum which was close to that of rhodoxanthin but never showed any fine structure (values for rhodoxanthin in parenthesis): 492 (490) nm in acetone, 492 (494) nm in ethanol, 482 (480) nm in *n*-hexane, 520 (520) nm in CS_2, and 499 (500) nm in benzene.

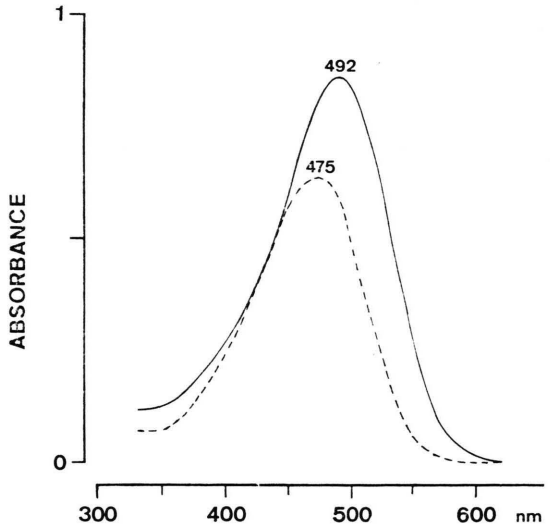

Fig. 2. Electronic spectra of 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione (continued line) and 2′-hydroxy-3,4-didehydro-β,β-caroten-2-one (dashed line) in acetone.

The infrared spectrum of the *Carausius* carotenoid (Fig. 3) exhibited a peak at 1655 cm^{-1} indicating conjugated carbonyl groups (C = O stretching), and, typically, a doublet in the *all-trans* region at 965 and 972 cm^{-1} due to C – H out-of-plane deformation vibrations of *trans*-disubstituted CH = CH-double bonds. Splitting of this absorption, which is normally represented as a singlet for *all-trans* carotenoids, is reported for *retro* and some keto carotenoids (*cf.* [17]). Other peaks were common

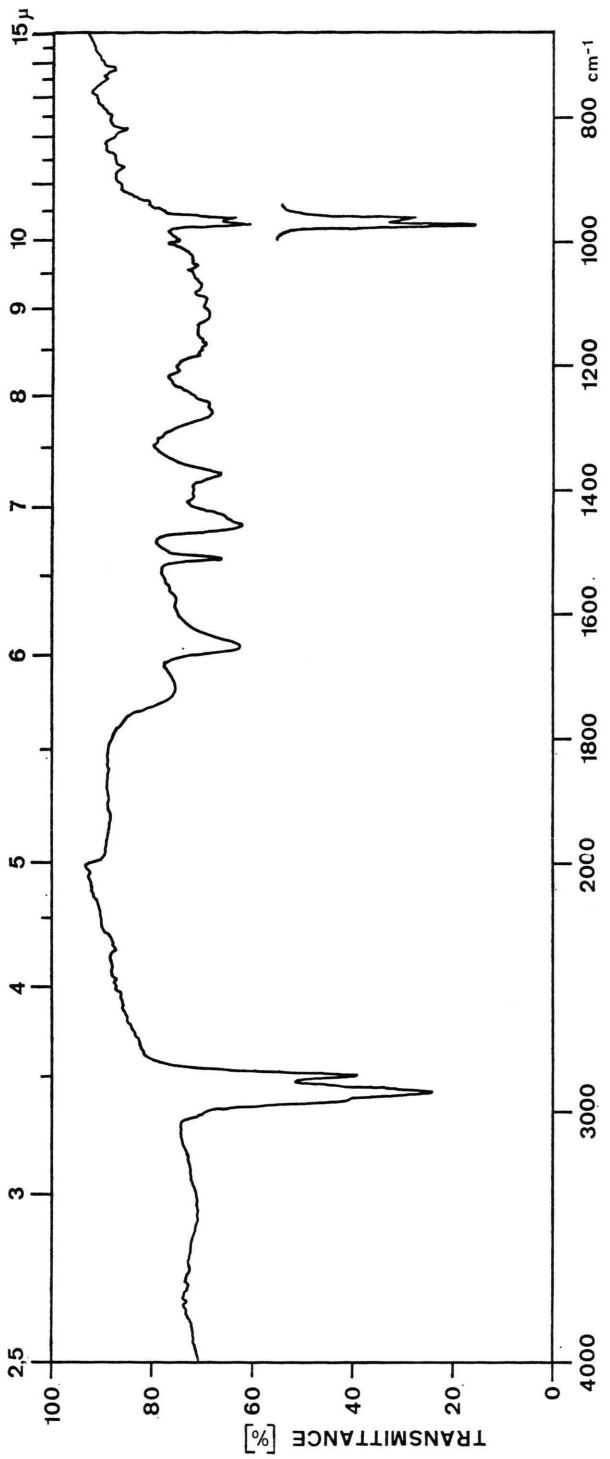

Fig. 3. Infrared spectrum (KBr) of 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione.

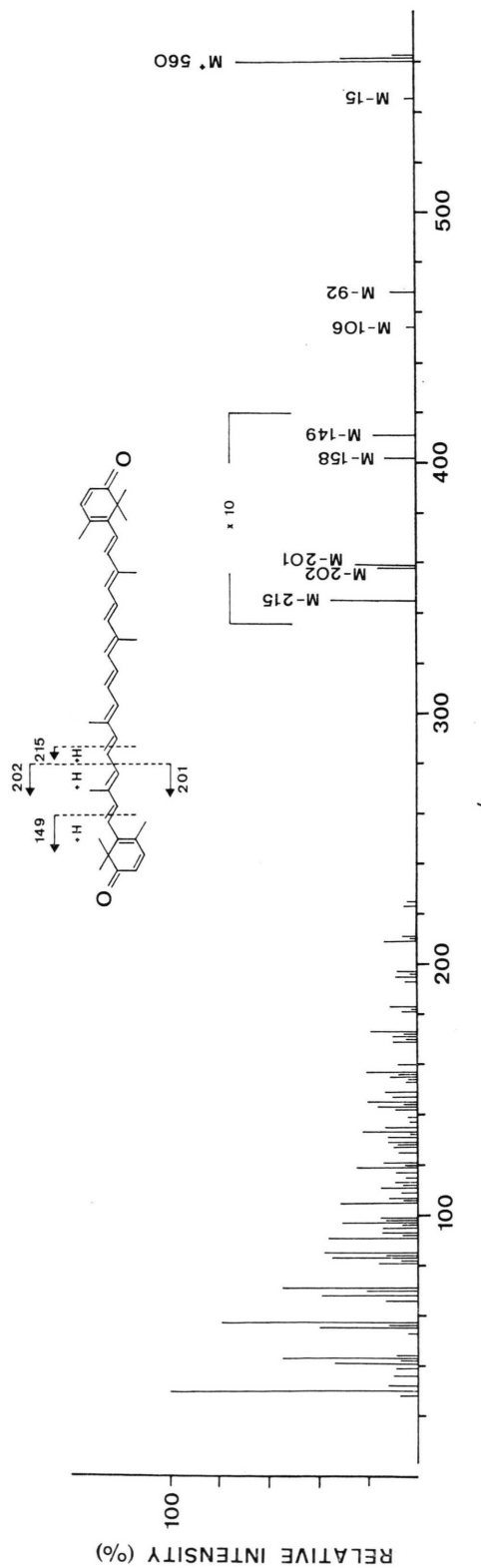

Fig. 4. Mass spectrum (70 eV; 8 kV; 180 °C) of 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione. $\times 10$ — peaks are magnified by factor 10.

C – H and C – C absorptions: 2955, 2850 cm^{-1} [CH_3], 2920 cm^{-1} [CH_2], 1455 cm^{-1} [CH_3 – C], 1375 cm^{-1} [$(CH_3)_2$ – C]. There were no O – H or C – O absorptions. Conclusively, all oxygen functions of this carotenoid should be represented as carbonyl groups conjugated to the polyene system.

The mass spectrum of the keto carotenoid (Fig. 4) exhibited a prominent molecular ion at *m/e* 560.3659 corresponding to $C_{40}H_{48}O_2$ (calcd 560.3654). Ions at *m/e* 468 (M-92), 454 (M-106), and 402 (M-158) were due to losses of toluene, xylene, and of a $C_{12}H_{14}$ fragment, respectively, from the polyene chain as typical for carotenoids [18]. The intensity ratio of M-92/M-106 ions was 3.1 and within the range observed for bicyclic carotenoids with nine conjugated double bonds in the chain [19]. The ion at *m/e* 411 (M-149), resulting from elimination of a $C_{10}H_{13}O$ fragment, as established by high precision measurements, was attributed to cleavage of the 7,8(7′,8′)-double bond with hydrogen transfer to the smaller fragment. The analogous rupture of the 11,12(11′,12′)-double bond was observed at *m/e* 345 (M-215) corresponding to loss of $C_{15}H_{19}O$. Similarly, peaks at *m/e* 358 (M-202) and 359 (M-201) were due to rupture of the 10,11(10′,11′)-single bond once with hydrogen transfer (loss of $C_{14}H_{18}O$) and once without this shift to the uncharged fragment (loss of $C_{14}H_{17}O$). According to the fragment ions, the carotenoid molecule should be symmetrical with oxygen functions in both end rings; however, since no fragmentation within the end groups themselves could be observed, the exact positions of oxygen atoms remained to be derived from other data. If compared with cleavage of the 7,8-double bond in canthaxanthin for example (M-151) (*cf.* [18]) the fragment lossed from the *Carausius* pigment contained two hydrogen atoms less (M-149); consequently, an additional C = C double bond is to be postulated for the end groups of the novel carotenoid. In the 12 eV spectrum only the molecular ion was observed (*m/e* 560).

Attempts to acetylate and silylate the red *Carausius* carotenoid were both unsuccessful; the pigment was recovered unchanged in both experiments. This negative result on hydroxyl groups is in agreement with the infrared spectrum according to which the presence of conjugated carbonyl groups was suggested. Reduction of the red pigment with $NaBH_4$ in ethanol or ethanol/methanol (*cf.* [10]) proceeded

very quickly within few minutes and yielded one intermediate orangered fraction ($\lambda_{max} = 479$ nm in acetone) and a final product zone orange in colour ($\lambda_{max} = 469$ nm in acetone) when chromatographed on silica gel-G. The depression of hRf-value from 34 to 24 for the fully reduced pigment, which co-chromatographed with β,β-carotene-2,2′-diol on silica gel-G, suggested the formation of two hydroxyl groups; chemically, this was confirmed by the formation of a diacetate (hRf = 68).

Rechromatography by multiple development of the fully reduced pigment fraction from silica gel-G on the adsorption plate revealed three distinct zones (Figs 5, 6B): 1. the redorange lowest zone was most prominent and exhibited a symmetrical electronic spectrum with λ_{max} at 471 in acetone; 2. the middle zone was yelloworange with an asymmetrical light absorption maximum at 462 nm in acetone; 3. the upper yellow fraction was low in quantity and showed the typical two-peaked electronic spectrum of β,β-carotene ($\lambda_{max} = 452$, 478 nm in acetone). This multiplicity of products was not dependent on the time of reduction, but, to a certain extent, one the solvent. If the reaction was carried out in ethanol the upper yellow zone could hardly be detected; however, in the presence of methanol (*cf.* [10]) the formation of the upper and medium fractions was favoured.

Regarding the main reduction product its electronic spectrum agreed exactly with that of synthetic

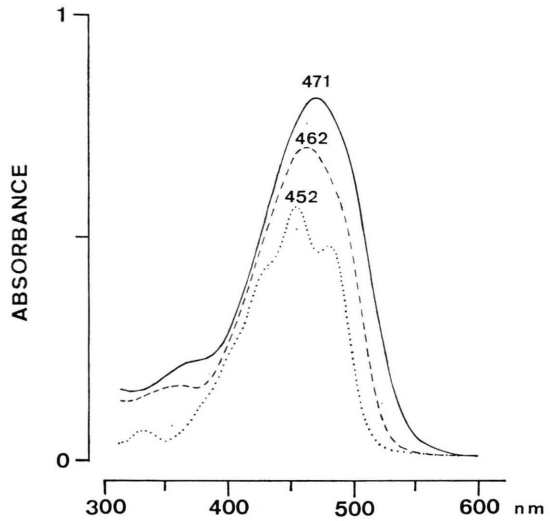

Fig. 5. Electronic spectra of the hydride reduction products of 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione in acetone after separation by adsorption TLC.

3,4,3′,4′-tetradehydro-β,β-carotene in respect to symmetry and position (see Methods). According to the hypsochromic shift of 21 nm and to the formation of two hydroxyl groups during reduction the native pigment was assessed to have two carbonyls in conjugation to the polyene chain, which was in agreement with infrared data. Consequently, the red *Carausius* pigment was assigned to 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione. For the cor-

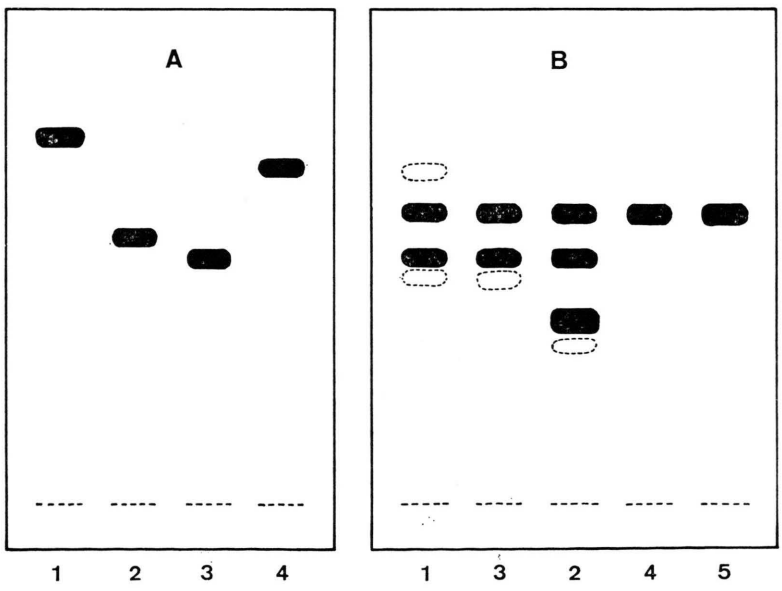

Fig. 6. Adsorption chromatograms (3× developed) of (A) native and (B) hydride reduced carotenoids.
(1) 3,4-Didehydro-β,β-carotene-2,2′-dione,
(2) 3,4,3′,4′-Tetradehydro-β,β-carotene-2,2′-dione,
(3) 2′-Hydroxy-3,4-didehydro-β,β-caroten-2-one,
(4) 2′-Hydroxy-β,β-caroten-2-one.
(5) Native β,β-carotene-2,2′-diol as reference.

responding reduction product (lowest fraction with $\lambda_{max} = 471$ nm) a molecular weight of 564 was expected. The mass spectrum (70 eV, 190 °C) of this compound, however, exhibited no peak at *m/e* 564 but a prominent ion at *m/e* 528 corresponding to a M-18-18 fragment. The common losses of toluene and xylene from this didehydrated ion were observed at *m/e* 436 (M-18-18-92) and 422 (M-18-18-106). The peak at *m/e* 395 was due to loss of 133 m.u. from the M-18-18 peak and was attributed to cleavage of the 7,8(7′,8′)-double bond with hydrogen transfer to the smaller fragment. In addition, a strong peak at *m/e* 133 was present caused by the same rupture with charge retention by the smaller fragment. Those ions are reported to be of diagnostic value for carotenoids with aromatic end groups [18], which in the pigment under investigation should have been formed from the 3,4-didehydro-2-ol end rings by elimination of the allylic hydroxyls and migration of one of the methyl groups at C-1(1′).

As to the medium product its asymmetrical absorption spectrum ($\lambda_{max} = 462$ nm) coincided with that of authentic 3,4-didehydro-β,β-carotene (see Methods). The native pigment, therefore, should be 3,4-didehydro-β,β-carotene-2,2′-dione. After hydride reduction the molecular weight is 566. In the mass spectrum (70 eV, 180 °C) of the reduced compound no molecular ion was found but a strong peak at *m/e* 548 corresponding to a M-18 fragment. Extrusions of toluene and xylene gave rise to peaks at *m/e* 456 (M-18-92) and 442 (M-18-106). In analogy to the compound discussed above dehydration should have taken place in that end ring with the hydroxyl in allylic position to the additional double bond a C-3,4. In agreement with this a fragment ion at *m/e* 415 (M-18-133) was observed which resulted from elimination of an aromatic end ring caused by cleavage of the 7,8-bond; the *m/e* 133 peak was present, too. As expected the corresponding rupture of the 7′,8′-bond was observed at *m/e* 395 (M-18-153) due to loss of a monohydroxylated β-end ring. Also the 12 eV spectrum did not show the true molecular ion expected at *m/e* 566 but a prominent M-18 fragment, suggesting that dehydration resulted from thermal degradation and not from electron impact.

The upper yellow reduction product (*cf.* Figs 5, 6 B) with the chromophoric system of β,β-carotene was identical with β,β-carotene-2,2′-diol on the adsorption plate.

According to the number of allylic hydroxyl groups in the main reduction product 3,4,3′,4′-tetradehydro-2,2′-diol this compound was easily converted into a diether in acidic ethanol; 3,4-didehydro-2,2′-diol yielded only a mono-ether (*cf.* [20]) as judged from the relative increments of Rf-values and from unchanged electronic spectra.

Since the red *Carausius* carotenoid had been highly purified by multiple development in the adsorption system (see below) all three final reduction products should be derived from 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione. This was further established by half-reduction with $NaBH_4$ in ethanol (Fig. 7). The transient zone, corresponding to formation of one hydroxyl only, was isolated from silica gel-G. Adsorption chromatography of this zone yielded two products: the lower one exhibited a symmetrical absorption spectrum with $\lambda_{max} = 482$ nm in acetone and corresponded to 2′-hydroxy-3,4,3′,4′-tetradehydro-β,β-caroten-2-one; the less adsorbed pigment, low in quantity, showed an asymmetrical peak at 475 nm in acetone and was assigned to 2′-hydroxy-3,4-didehydro-β,β-caroten-2-one. The 482 nm-pigment was further hydride reduced and subsequently subjected to adsorption

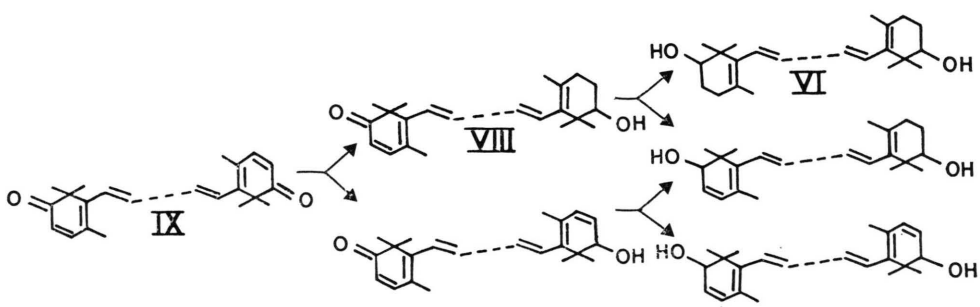

Fig. 7. Scheme of hydride reduction of 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione (**IX**). Products **VIII** and **VI** are natively present in *C. morosus* (*cf.* Figs 1, 9).

TLC. Two products (Fig. 7), roughly equal in quantity, were found showing λ_{max} at 472 and 462 nm (acetone), respectively, which co-chromatographed with the corresponding reduction products discussed above. This means, that actually one 3,4-double bond was hydrogenated during hydride reduction. Since the reduced dehydro products were not changed when further treated with $NaBH_4$, hydrogenation of the C = C-double bond seems to be closely connected with the reduction of the conjugated carbonyl group.

Conclusively, results of hydride reduction experiments should be very carefully interpreted in those keto carotenoids under investigation. The two diketones corresponding to both additional reduction products of the red *Carausius* pigment could be isolated, too, but were already separated from the tetradehydro compound on silica gel-G (*cf.* Fig. 1). Only one minor zone, obviously a *cis* isomer of the tetradehydro-dione, could be separated from the main pigment fraction by multiple development as a result of its slightly reduced adsorption. The electronic spectrum was blue-shifted to $\lambda_{max} = 484$ nm in acetone. While standing in solution the isomer was partly converted to the *all-trans* compound. On reduction, additionally to the known products ($\lambda_{max} = 471$ and 461 nm, respectively) the corresponding *cis*-isomers could be discerned due to their increased polarity ($\lambda_{max} = 466$ and 457 nm, respectively, in acetone).

2′-Hydroxy-3,4-didehydro-β,β-caroten-2-one (**VIII**)

This red carotenoid was a minor fraction which, according to saponification results, mainly occurred as a mono-ester (hRf = 39; Fig. 1). The free pigment present in low amounts in the original extract (hRf = 21) ran between the tetradehydro-dione and 2,2′-diol suggesting the presence of one hydroxyl and one carbonyl group. After purification by adsorption TLC of the saponified pigment one symmetrical absorption maximum was found at 474 – 476 nm in acetone (Fig. 2).

The native ester was investigated by mass spectrometry (a minor zone was separated by adsorption TLC of the silica gel-G fraction). A prominent molecular ion (Fig. 8) was found at *m/e* 826 corresponding to $C_{58}H_{82}O_3$. A second strong peak was at *m/e* 546 ($C_{40}H_{50}O$). The difference of 280 m.u. ($C_{18}H_{32}O_2$) was due to elimination of a 18:2 fatty acid, most probably linoleic acid. Eliminations of toluene and xylene, respectively, were observed at *m/e* 734 (M-92), 720 (M-106), 454 (M-280-92), and 440 (M-280-106). Two other fragment ions revealed structural features of the carotenoid. The peak at *m/e* 411 (M-415 = M-280-135) corresponding to $C_{30}H_{35}O$ was attributed to cleavage of the 7′,8′-double bond with hydrogen transfer to the uncharged fragment and elimination of the esterified ring. From the data presented it can be calculated that in the free carotenoid this end group is a monohydroxy cyclohexene ring. The composition of

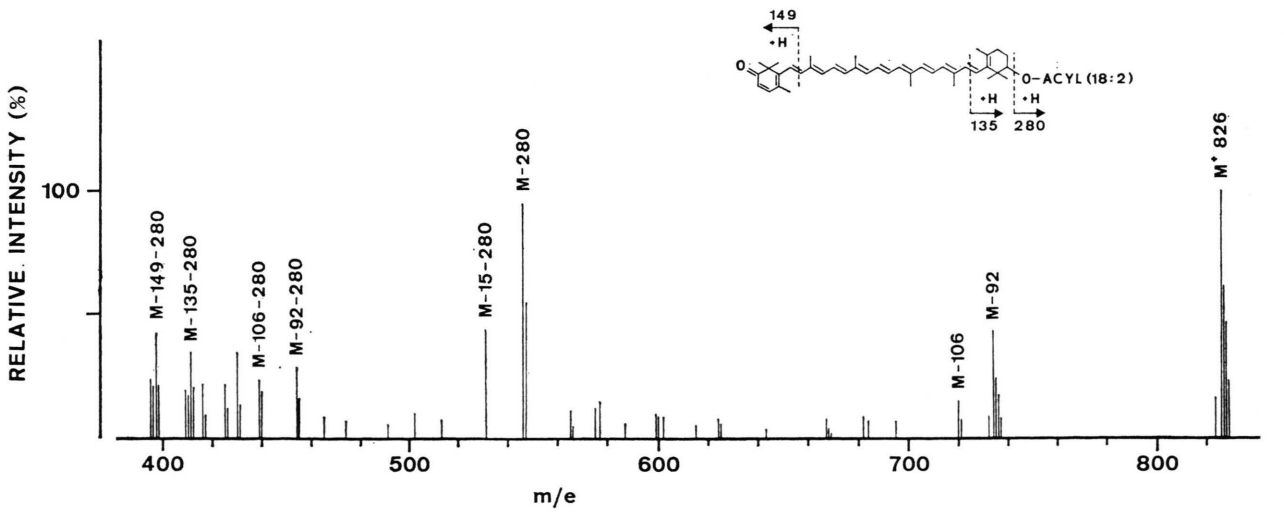

Fig. 8. Mass spectrum (70 eV; 6 kV; 200 °C) of the linoleic acid (18 : 2) ester of 2′-hydroxy-3,4-didehydro-β,β-caroten-2-one.

the *m/e* 411 fragment was identical to that of the ion resulting from the corresponding rupture in 3,4,3′,4′-tetradehydro-β,β-carotene-2,2′-dione (*cf.* Fig. 4; M-149) suggesting structural identity of one of the end rings in both pigments. This was confirmed by the presence of the *m/e* 397 peak (M-429 = M-280-149) which corresponded to cleavage of the 7,8-bond in the deacylated pigment. Conclusively, the free carotenoid alcohol should have a molecular weight of 564 ($C_{40}H_{52}O_2$). This was actually found for the saponified pigment.

The hydride reduction product of the free carotenoid behaved similar to β,β-carotene-2,2′-diol on silica gel-G. On adsorption TLC two fractions were obtained which co-chromatographed with the two side-products of reduced tetradehydro-dione (Fig. 6 B) and exhibited also spectral identity with those: the main product had an asymmetrical peak at 462 nm in acetone, the minor fraction showed a β-carotene-chromophore with λ_{max} at 452 nm and behaved identical to 2,2′-diol. Obviously, this minor product was a result of 3,4-hydrogenation in the course of reduction of the conjugated carbonyl group.

Consequently, the structure of this red pigment was assigned to 2′-hydroxy-3,4-didehydro-β,β-caroten-2-one, which, artificially, could be produced as a side-product during half-reduction of the tetradehydro-dione (*cf.* Fig. 7); the electronic spectra of both compounds were identical (λ_{max} = 475 nm in acetone).

3,4-Didehydro-β,β-carotene-2,2′-dione (**VII**)

On overloaded silica gel-G chromatograms this minor red carotenoid (hRf = 30) was completely masked by the mono-ester fraction of 2,2′-diol. Purification was easily performed by acetylation of the contaminating hydroxylated pigments. The chromatographic properties of this red carotenoid were not affected by the procedure. It behaved less polar than tetradehydro-dione in both systems (Figs 1, 6 A).

The electronic spectrum coincided with that of 2′-hydroxy-3,4-didehydro-2-one (λ_{max} = 475 nm in acetone) demonstrating identical chromophoric systems. Furthermore, the products of hydride reduction were identical in both pigments when compared on silica gel-G and on the adsorption layer (Fig. 6 B). Their electronic spectra were superimposable, too: the main product with an asymmetrical peak at 462 nm, and the minor compound, less adsorbed, with a β-carotene-type spectrum (λ_{max} = 451 nm). Due to lack of material no mass spectrum could be recorded. Nevertheless, the structure of 3,4-didehydro-β,β-carotene-2,2′-dione was evident.

2′-Hydroxy-β,β-caroten-2-one (**V**)

Upon saponification the native yellow fraction (hRf = 45) yielded – without any intermediate – a more polar product (hRf = 26; Fig. 1), which behaved slightly more polar than the tetradehydro-dione on silica gel-G but less polar on silica gel 60 HR; the latter behaviour was more pronounced on the adsorption plate (Fig. 6 A). The saponified product yielded a mono-acetate, which ran below the native fraction suggesting a long chain fatty acid in the naturally esterified pigment.

The electronic spectrum demonstrated the chromophoric system of β,β-carotene with maxima at *450* and 477 nm in acetone (% **III/II** = 17). Mass spectrometry (70 eV; 185 °C) revealed a strong molecular ion at *m/e* 566.4141 corresponding to $C_{40}H_{54}O_2$ (calcd 566.4122). This was the only ion in the 12 eV spectrum. Common fragmentations were observed at *m/e* 474 (M-92) and 460 (M-106); the ratio of toluene to xylene elimination (3.6) was in agreement with nine double bonds in the polyene chain [19].

From the molecular weight a didehydro derivative of β,β-carotene-2,2′-diol was suggested. Since one oxygen function is a hydroxyl group, as established chemically, the second one should be a carbonyl function. This was confirmed by hydride reduction which yielded a more polar product identical with the 2,2′-diol on adsorption TLC (Fig. 6 B). The native compound, therefore, is 2′-hydroxy-β,β-caroten-2-one.

Recently, a specific reaction of β,β-caroten-2-ol and β,β-carotene-2,2′-diol in acidic solution was found leading to keto products with *retro*-shifted polyene systems [6, 10, 21]. It was interesting to test the reaction of the compound under investigation. However, treatment with 0.1 M BF_3-etherate in chloroform (30 min in air) yielded a variety of yellow and red products, most of them being more polar than the original carotenoid on silica gel-G. One of them exhibited the same *retro*-shaped electronic spectrum (λ_{max} = 419, *443*, 472 nm in acetone) as the final product of the diol (*cf.* [6]), but was quite different on TLC. This indicates, that

reactivity in both end rings is required for this specific conversion.

β,β-Caroten-2-one (**II**) and *β,β-Carotene-2,2′-dione* (**IV**)

These two compounds were trace pigments, which could only be isolated from large-scale preparations. The 2-one was found between the diesters of 2,2′-diol and the ester of 2′-hydroxy-2-one (hRf approx. 50). The electronic spectrum was β-typed (λ_{max} = 450). Hydride reduction revealed a product which co-chromatographed with β,β-caroten-2-ol on silica gel-G. The 2,2′-dione is, up to now, very tentatively identified by its expected relative mobility (hRf approx. 35) and yellow colour. The traces available were not sufficient for reduction experiments.

Discussion

The 2-keto carotenoids isolated from *Carausius* have been neither isolated from a biological source nor synthesized by chemical methods before. So it was quite surprising to find pigments of this type in an insect, since animals, generally, are known to be not as good carotenoid chemists as plants or bacteria (*cf.* [22]).

All ketonic and hydroxylated derivatives of β-carotene isolated from *Carausius* are actually biosynthesized in this insect as shown by feeding labelled β-carotene [23]. Recently, the transformation of carotenoids by insects has been demonstrated by tracer experiments for the first time [24]. Apart from low amounts of lutein, β-carotene used as precursor is selectively absorbed by the insect from a variety of carotenoids present in the food [5]. The question arises in which sequence the various pigments are synthesized in *Carausius*. When in an organism both hydroxy and keto carotenoids of the same substitution type are found, the proposed pathway commonly involves the hydroxy compounds as intermediates in the formation of the keto pigments from the carotene precursor (*cf. e.g.* [25, 26]). This seems to be reasonable from a chemical view and is also accepted for *Carausius*. However, according to the pathway tentatively proposed for the carotenoid metabolism in the stick insect as shown in Fig. 9, the product of the hydroxylation step should occur only as a transient state within the enzyme complex and not been liberated; immediately, the hydroxy compound is dehydrogenated to the corresponding ketone as suggested by Davies *et al.* [27] for the metabolism of β-carotene in the brine shrimp *Artemia*. The hydroxylated carotenoids in *Carausius*, present in relative large amounts, are suggested to be reduction products of the ketones and, consequently, should not serve as precursors of the tetradehydro-dione **IX** as the predominant red metabolite. So the hydroxy compounds appear as side products, and this could explain their accumulation and esterification with fatty acids. According to previous studies [8, 28] hydroxylated carotenoids are predominantly bound to polyunsaturated fatty acids, which are essential factors for development, sug-

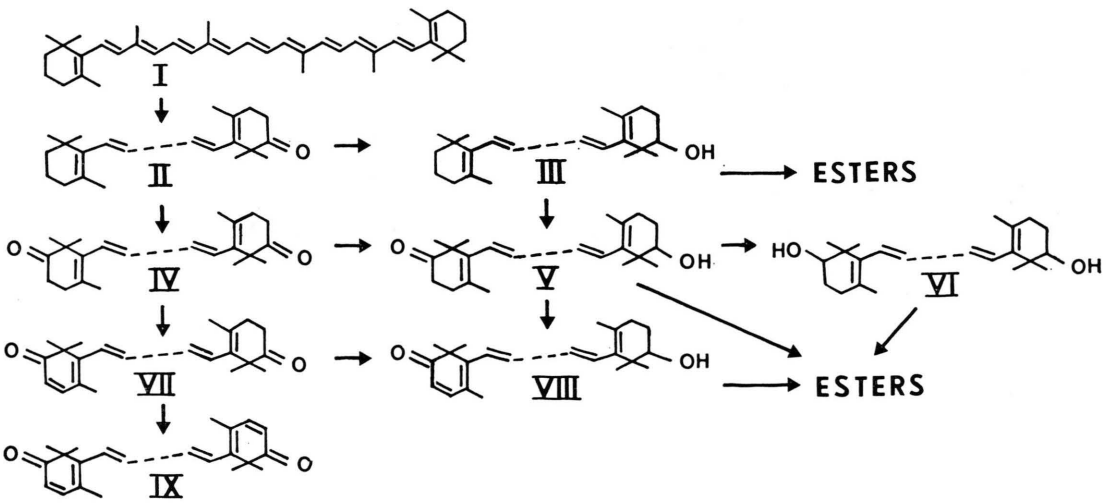

Fig. 9. Proposed pathway of carotenoid metabolism in *C. morosus* (*cf.* Fig. 1).

gesting a storage function for the esters in lipid metabolism. In the only ester studied in *Carausius* a 18:2 fatty acid was found, which confirms this view.

The proposed metabolic pathway (Fig. 9) further accounts for the fact, that β,β-carotene-2,2′-diol (**VI**) is the main carotenoid in the stick insect (*cf.* [5]); the 3,4-didehydro pigments **VII** and **VIII** including the end product **IX** are present in lower amounts, indicating that introduction of this C = C double bond takes place to a minor extent than reduction of a keto group. The trace amounts of the ketones **II** and **IV** are fitting well into this scheme; since they serve as precursors in two types of reactions, dehydrogenation and reduction (hydrogenation), rapid turnover rates and small pool sizes are expected. Compounds with 3,4-didehydro-2-ol end rings could not be detected in *Carausius* suggesting that dehydrogenation of the C – C bond does not occur in hydroxylated rings, and that conjugated keto groups are not reduced to hydroxyls.

Generally, though still a hypothetical scheme, the proposed carotenoid pathway properly explains quantitative and qualitative data on the *Carausius* carotenoids including labelling results so far obtained [23]. This is in contrast to an alternative possible pathway which is based on the assumption that the hydroxy pigments are converted to ketones. This view, commonly held in the carotenoid field, can not be ruled out in *Carausius* presently. A detailed study with labelled β-carotene, now under way, will bring final evidence on the mechanism of carotenoid transformation in the stick insect.

The technical assistance of Mrs. B. Zorn is gratefully acknowledged. The mass spectra were recorded by the courtesy of Dr. Prox from Dr. K. Thomae Ltd., Biberach. Insects were kindly supplied by Prof. D. Bückmann, this department, and Dr. G. Berthold, University of Giessen. I am grateful to Prof. S. Liaaen-Jensen, University of Trondheim, for valuable discussions and for her interest in this work. Finally, I would like to thank F. Hoffmann-La Roche, Basel, for authentic canthaxanthin, and the Deutsche Forschungsgemeinschaft for financial support (SFB 87).

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