# Isolation of β, β-Caroten-2-ol from an Insect, *Cerura vinula* (Lepidoptera)

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Identification of 2-Hydroxy Carotenoids, *β,β*-Caroten-2-ol, Acid Catalyzed Dehydrogenation, Insect Carotenoids, *Cerura vinula*

The monohydroxy carotenoid from the moth *Cerura vinula* has been shown to be *β,β*-caroten-2-ol on the basis of electronic, infrared, proton magnetic-resonance, and mass spectra. On acid treatment in the presence of molecular oxygen this carotenoid is dehydrogenated to 4′,5-*retro-β,β*-caroten-2-one. The identification of *β,β*-caroten-2-ol by its *retro* product, its time course of acetylation, and its chromatographic properties relative to *β,β*-caroten-3-ol and *β,β*-caroten-4-ol is discussed.

This is the first demonstration of a 2-hydroxylated carotenoid in an insect. Implications on the biogenesis of this pigment are considered.

## Introduction

The carotenoids and their contribution to the colour change[1] in the larva of the moth *Cerura vinula* were studied by Nagel[2]. Thereby, the occurence of a monohydroxy carotenoid was reported, which amounted to about 40% of the carotenoids present in the integument and haemolymph of last instar larvae. As shown by co-chromatography this pigment was not identical to *β,β*-caroten-3-ol (cryptoxanthin) or the corresponding 4-ol (isocryptoxanthin).

The structure of this carotenoid has now been established by means of spectroscopic methods and shown to be *β,β*-caroten-2-ol. Recently, this pigment and other 2-hydroxylated carotenoids have been found in a green alga for the first time in nature[3]. The presence of this carotenoid in certain crustacea was stated by co-chromatography with the authentic algal pigment[4]. The isolation of *β,β*-caroten-2-ol from *Cerura* is the first one reported for insects. During this work the 2-ol was also found in an orthopteran species together with other structurally related carotenoids[5, 6].

A short communication on the chemical behaviour of 2-hydroxylated carotenoids has been given previously[7].

## Materials and Methods

### Insects

*Cerura vinula* (Notodontidae) was obtained from several sources. Pupae were purchased from German entomologists and from Worldwide Butterflies Ltd. (Sherborne Dorset, England). The larvae were fed on leaves of *Populus nigra*. Only pupae and adult insects were used for carotenoid isolation.

### Isolation of carotenoids

The methods of carotenoid extraction and separation by thin-layer chromatography (TLC) were the same as published previously[8]. After two runs in the silica gel-G partition system with a mixture of petroleum ether (100 — 140 °C) and propanol-2 (130:8; v/v) as solvent the pigments were finally purified by adsorption TLC on $CaCO_3/MgO/Ca(OH)_2$ plates[9] using a solvent of petroleum ether, acetone, and chloroform (130:5:5; v/v/v) or, preferably, the solvent of the silica gel-G system. For analytical purposes reversed-phase partition TLC on paraffine impregnated cellulose[8] with methanol, acetone, and water (20:10:1; v/v/v) as solvent, and TLC on polyamide[10] with petroleum ether and methyl ethyl ketone (100:0.5; v/v) were applied in addition. Crystallization of the mono-ol was achieved from petroleum ether (50 — 70 °C)-methanol.

### Chemical treatments

Saponification was performed with 3% Na-methylate in methanol (w/w), or with 5% KOH in methanol (w/w) at room temperature. — Acetylations of hydroxyls were done with acetic anhydride

in dry pyridine (1:5; v/v). Time courses of acetylations were studied as reported in a preceding paper [11]. — Acid treatments of carotenoids for etherification or dehydration were done with $BF_3$-etherate in ethanol or chloroform, respectively. Solutions were made up to 0.1 M of $BF_3$ [12]. For experiments with HCl/chloroform dry HCl gas was passed through commercial chloroform to obtain a stem solution which was diluted with neutral chloroform in varying amounts (1:10, 1:100, etc.) for use. The reactions were stopped by vigorous shaking with water. — Oppenauer oxidations were performed for $3-5$ h using acetone and aluminium-isopropanolate after the method of Hager and Stransky [13]. — Reductions of carbonyl groups were mostly carried out with $NaBH_4$ in ethanol or in ethanol/methanol (4:1; v/v). (In the presence of methanol the more powerful $NaBH(OCH_3)_3$ is formed [14].) $LiAlH_4$ was used in dry re-destilled diethyl ether. Reactions were allowed to proceed at room temperature for several hours.

### Reference carotenoids

Isocryptoxanthin ($\beta,\beta$-caroten-4-ol) was synthesized either by borohydride reduction of authentic echinenone ($\beta,\beta$-caroten-4-one), or from $\beta,\beta$-carotene by hydrolytic cleavage of its $BF_3$-complex [15]. The ethyl ether (4-ethoxy-$\beta,\beta$-carotene) was obtained on treatment of the 4-ol with $BF_3$/ethanol as described above. The dehydration to 4′,5′-didehydro-4,5′-retro-$\beta,\beta$-carotene was performed with $BF_3$/chloroform. Cryptoxanthin ($\beta,\beta$-caroten-3-ol) was isolated from ripe fruits of red pepper (*Capsicum annuum*) [16]. Additionally, authentic cryptoxanthin was kindly supplied by Hoffmann-La Roche (Basel). Synthetic $\beta,\beta$-carotene was purchased from Merck (Darmstadt, Germany).

### Spectroscopy

Electronic spectra were recorded on a Zeiss spectrophotometer type DMR 21 using 1 cm glass cuvettes. An extinction coefficient of $E_{1cm}^{1\%} = 2300$ ($cf$ [3]) was assumed for the mono-ol ex *Cerura*. — Infrared spectra were obtained on a Perkin-Elmer 325 spectrometer. For this, the pigments in $CCl_4$ solution were spread on a KBr or CsJ disc and, after evaporation of the solvent, covered with a second disc. — Mass spectrometry was performed with a Varian Mat CH5 spectrometer or an AEI MS902S instrument using the direct inlet systems. The spectra were recorded at 170 °C with electron impacts of 70 eV. Whenever possible 12 eV spectra were run in addition. Perfluorokerosene was used as reference. The preparation of samples for mass spectrometry has

been described previously [8]. — The NMR spectrum was recorded on a Bruker HX-90 instrument in $CDCl_3$ solution relative to tetramethylsilane (TMS) as internal standard. The spectrum was obtained by the Fourier transform technique; 12740 pulse interferograms were accumulated.

### Chemicals and chromatographic materials

Chemicals were obtained from Merck (Darmstadt, Germany) if not stated otherwise. If available purity was of analytical grade. Na-methylate, $BF_3$-etherate, and $NaBH_4$ were for laboratory use. Pyridine was dried over KOH platelets. $LiAlH_4$ was a product of Fluka (Buchs, Switzerland). Precoated layers of cellulose (F 1440) and of polyamide (G 1600) were obtained from Schleicher and Schüll (Dassel, Germany).

### Nomenclature

The new carotenoid nomenclature, recommended by IUPAC [17], is used in this paper besides trivial names.

## Results and Discussion

The silica gel-G chromatogram of the unsaponified carotenoid extract from *C. vinula* (Fig. 1) shows
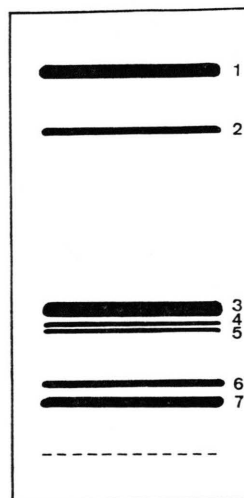


Fig. 1. Silica gel-G chromatogram of the unsaponified carotenoid extract from *Cerura vinula*. (1) $\beta,\beta$-carotene; (2) diesters of lutein; (3) $\beta,\beta$-caroten-2-ol; (4, 5) monoesters of lutein and zeaxanthin; (6) 3′-dehydrolutein; (7) lutein+zeaxanthin.

the presence of $\beta$-carotene ($\beta,\beta$-carotene), lutein ($\beta,\varepsilon$-carotene-3,3′-diol), and the monohydroxy carotenoid as reported by Nagel [2]. Other zones represent lutein diesters, monoesters of lutein and zeaxanthin ($\beta,\beta$-carotene-3,3′-diol), and 3′-dehydrolutein, the identification of which has been reported recently [11]. The monohydroxy carotenoid has been shown to be

2-hydroxylated $\beta$-carotene ($\beta,\beta$-caroten-2-ol) from spectroscopic, chromatographic and chemical data.

## The native pigment

The electronic spectrum (Fig. 2A) of the purified $\beta,\beta$-caroten-2-ol exhibited maxima at 452 and
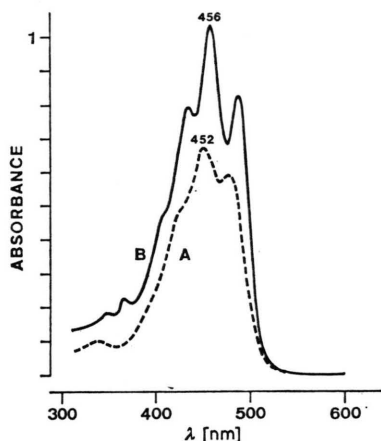
Fig. 2. Electronic spectra (acetone) of $\beta,\beta$-caroten-2-ol (A) and of its *retro* product obtained by acid treatment (B).

479 nm in acetone (% III/II = 22), at (426), 448, and 475 nm in hexane (% III/II = 35), and at 480 and 509 nm in $CS_2$ (% III/II = 12), demonstrating the chromophore of $\beta,\beta$-carotene. (The % III/II value indicates the height of the long-wave peak relative to the main absorption peak if the minimum between both is taken as the base [18].)

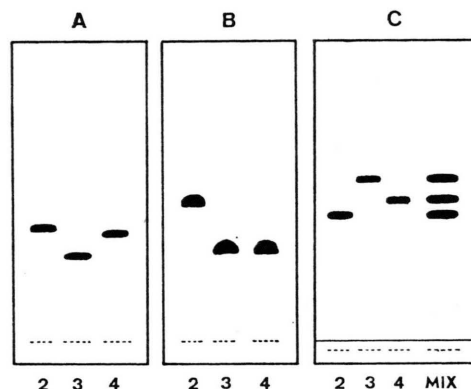During co-chromatography of $\beta,\beta$-caroten-2-ol with $\beta,\beta$-caroten-3-ol (cryptoxanthin) and $\beta,\beta$-caro-

Fig. 3. Chromatograms on silica gel-G (A), on the adsorption layer ($CaCO_3/MgO/Ca(OH)_2$) (B), and on paraffine impregnated cellulose (C) of monohydroxy carotenoids. (2) $\beta,\beta$-caroten-2-ol; (3) $\beta,\beta$-caroten-3-ol; (4) $\beta,\beta$-caroten-4-ol; (MIX) mixture of all three mono-ols.

ten-4-ol (isocryptoxanthin) on silica gel-G (Fig. 3A) the 3-ol as the most polar one was well separated from the 2-ol and 4-ol, the latter of which ran just behind the 2-isomer. On the adsorption plate (Fig. 3B) the 3-ol and 4-ol could not be distinguished from each other, whereas the 2-ol was less strongly adsorbed. An excellent separation of all three isomeric mono-ols, however, was demonstrated by reversed-phase partition chromatography on impregnated cellulose (Fig. 3C).

The infrared spectrum of the 2-ol (Fig. 4A) did not provide specific structural features. Besides the common CH absorptions [2955, 2850 cm$^{-1}$: $CH_3$; 2920 cm$^{-1}$: $CH_2$; 1440 cm$^{-1}$: $CH_3-C$; 1353, 1390 cm$^{-1}$: $(CH_3)_2C$] the hydroxyl group gave rise to absorptions at $3300-3600$ cm$^{-1}$ (O–H
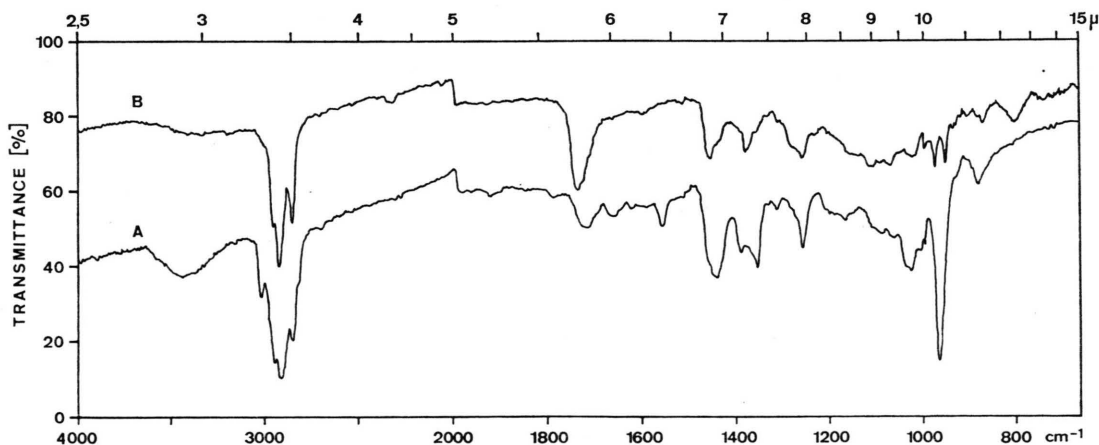
Fig. 4. Infrared spectra (KBr) of $\beta,\beta$-caroten-2-ol (A) and of its *retro* product obtained by acid treatment (B).
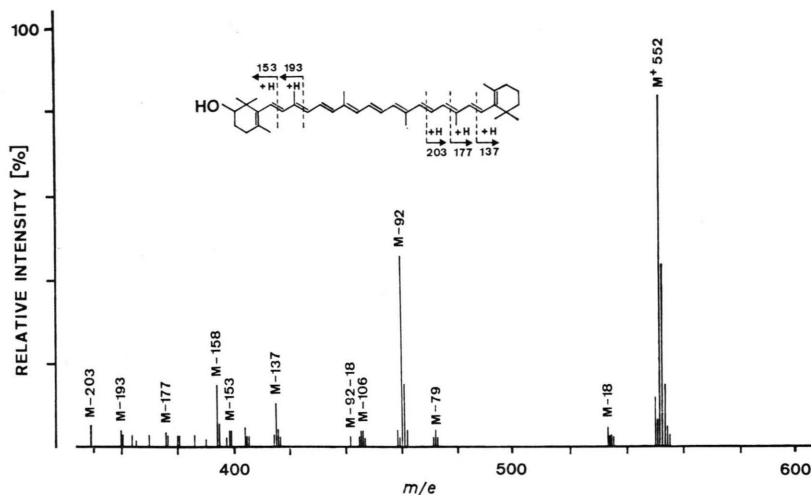
stretching) and $1020-1025$ cm$^{-1}$ (C $-$ O stretching or OH deformation). A sharp strong singlet at 963 cm$^{-1}$ was due to the all-*trans* disubstituted $-$ CH $=$ CH $-$ groupings (C $-$ H out-of-plane deformation). The corresponding stretching peak occured at 3020 cm$^{-1}$.

The mass spectrum of the *Cerura* pigment (Fig. 5) showed a prominent molecular ion at $m/e$ 552.4324 corresponding to $C_{40}H_{56}O$ (calcd. 552.4330). Its intensity was 83% of the base peak at $m/e$ 105. A small M-2 peak was found. Fragmentations common to the carotenoid polyene chain [19] were observed: loss of toluene ($m/e$ 460; M-92), of xylene ($m/e$ 446; M-106) of a $C_{12}H_{14}$ fragment ($m/e$ 394; M-158), and of $C_6H_7$ ($m/e$ 473; M-79). The origins of these fragments are known from deuterium labelled carotenoids [20, 21]. The intensity ratio of the M-92/M-106 ions (13.3) was within the range observed for β,β-carotene [22]. The presence of the hydroxyl group was confirmed by the loss of water ($m/e$ 534; M-18, and $m/e$ 442; M-92-18). The location of the hydroxyl in one of the end rings was assigned by the elimination of 153 m.u. (corresp. $C_{10}H_{17}O$) from the molecular ion arising from a cleavage of the 7,8-double bond ($m/e$ 399). Rupture of the 7′,8′-double bond resulted in a loss of 137 m.u. (corresp. $C_{10}H_{17}$) observed at $m/e$ 415. Peaks corresponding to cleavages of all further in-chain double bonds have been encountered being combined with hydrogen transfer to the uncharged fragment. In the 12 eV spectrum only the molecular ion and the M-92 fragment were observed.

The peracetylated carotenoid exhibited the molecular ion at $m/e$ 594 corresponding to a monoacetate.

The peaks at M-92 ($m/e$ 502) and M-158 ($m/e$ 436) could be well established but those at M-60 ($m/e$ 534) and M-106 ($m/e$ 488) were of low intensity.

Only the $^1$H NMR spectrum of the mono-ol gave substantial data on its 2-hydroxy structure (Fig. 6). A double doublet of signals at 3.57 ppm (1H) was due to vicinal coupling of the methine proton at C-2 carrying the hydroxyl group to the magnetically non-equivalent methylene protons at C-3. In the 3-ol and 4-ol the corresponding signals are shifted to a lower field [3]. As a result of the proximity of the hydroxyl group at C-2 to the *gem*-dimethyl group at C-1 the corresponding methyl signal was split into peaks at 1.09 ppm (singlet, 3H) and at 1.03 ppm (singlet, 9H). The signal of the end-of-chain methyls at C-5,5′ was found at 1.72 ppm (6H) as a singlet, which confirmed the presence of two β-rings and excluded the 4-ol structure in addition [23]. Other protons showed peaks at 1.97 ppm (singlet, 12H, in-chain methyls), 1.56 ppm (broad; 2′,3,3′-CH$_2$), 1.26 ppm (probably OH), and 6.06 $-$ 6.78 ppm (14H, olefinic protons). Generally, apart from the higher resolution this NMR spectrum of β,β-caroten-2-ol from an insect agrees very well with the spectrum of the 2-ol from a green alga [3]. The only difference was found in the coupling constants of the H-2 double doublet which were greater in the insect pigment ($J_1 \sim 12$ Hz, $J_2 \sim 6$ Hz) than in the algal one (7 and 4.5 Hz, respectively [3]). It is not known whether these data are related to different conformations of the two carotenoids. CD measurements may provide more information on this question.
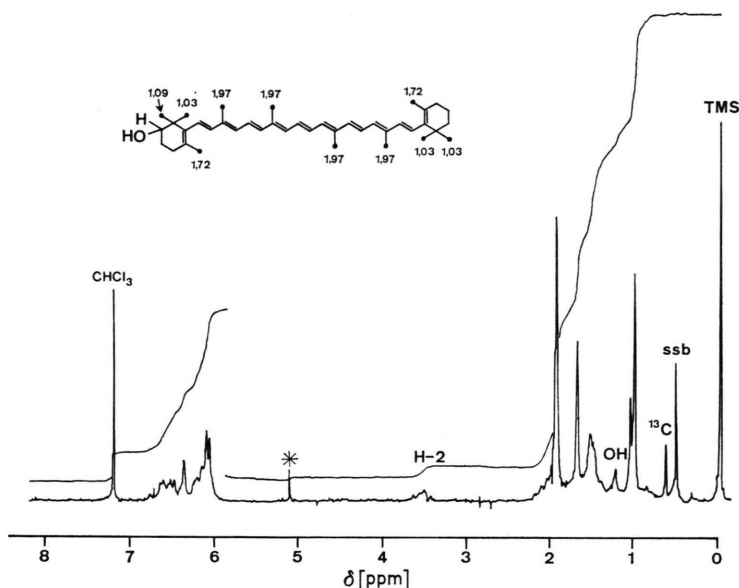
The reduced polarity of the β,β-caroten-2-ol if compared with the 4-ol and 3-ol isomers can be explained by a shielding effect of the *gem*-dimethyl group at C-1. Consequently, the time course of acetylation of the 2-hydroxyl group should be slower than in non-shielded hydroxyls. Comparisons were made with the non-allylic 3-OH and the allylic 3′-OH, respectively, of β,ε-carotene-3,3′-diol (lutein) using 3-hydroxy-β,ε-caroten-3′-one, and 3-acetoxy-β,ε-caroten-3′-ol[11]. As shown in Fig. 7 the three hydroxyl groups can be distinguished by their time courses of acetylation. As expected the reaction with the 2-ol proceeded slowest (50% acetate after 3.2 h) due to sterical effects. This is in close agreement with results on the algal 2-ol[24]. The 3′- and 3-OH

of lutein behaved similar to each other, however, the allylic 3′-hydroxyl was assigned by its significant slower time course of acetylation if compared with the non-allylic one (50% acetate after 68 and 50 min, respectively[11]).

## The acid-product

The β,β-caroten-2-ol under investigation showed no reaction during Oppenauer oxidation, no ether formation in acidic alcohols, and no elimination of the hydroxyl group upon treatment with acidic chloroform in contrast to β,β-caroten-4-ol. Under acidic conditions, however, the 2-ol exhibited a very specific reaction[7]. Treatment with 0.1 M BF$_3$-
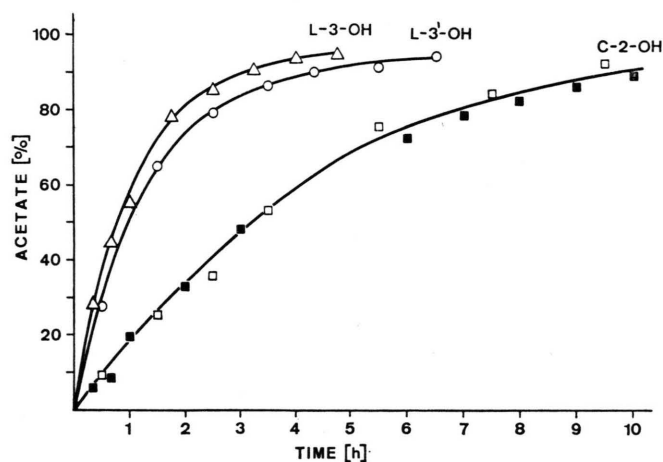


Fig. 7. Time courses of acetylations. L-3-OH — non-allylic hydroxyl of lutein; L-3′-OH — allylic hydroxyl of lutein; C-2-OH — β,β-caroten-2-ol. Black and white squares represent different experiments.

etherate in ethanol or chloroform, preferably, resulted in a less polar product, which co-chromatographed with the acetylated 2-ol both on silica gel-G and in the reversed-phase partition system (Fig. 8A, B). No intermediate was observed. The electronic
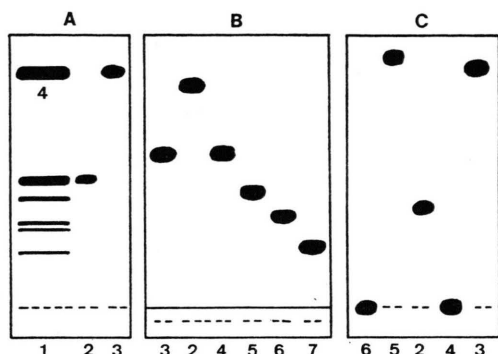


Fig. 8. Chromatograms of (1) products of BF₃/chloroform treatment of $\beta,\beta$-caroten-2-ol, (2) $\beta,\beta$-caroten-2-ol, (3) acetylated $\beta,\beta$-caroten-2-ol, (4) *retro* product of $\beta,\beta$-caroten-2-ol, (5) ethylated $\beta,\beta$-caroten-4-ol, (6) *retro* product of $\beta,\beta$-caroten-4-ol, and (7) $\beta,\beta$-carotene on silica gel-G (A), paraffine impregnated cellulose (B), and polyamide (C).

tronic spectrum of this compound (Fig. 2B) exhibited a slight bathochromic shift (432, 456, and 485 nm in acetone; % III/II = 40) and a typical *retro* shape similar to that of the dehydration product of $\beta,\beta$-caroten-4-ol (4′,5′-didehydro-4,5′-*retro*-$\beta,\beta$-carotene: 444, 470, 498 nm in acetone). From the spectral properties a chromophoric system consisting of eleven conjugated double bonds in *retro* position was deduced [5].

The infrared spectrum of the product of acid treatment (Fig. 4B) showed no absorptions in the OH-regions but a strong peak at 1735 cm⁻¹ typical for isolated carbonyl groups (C = O stretching); the all-*trans* singlet was replaced by a doublet at 953 and 976 cm⁻¹ reported to be significant for *retro* carotenoids [23]. This would mean a conversion of the hydroxyl to a carbonyl group and not its elimination.

The mass spectrum of the acid-product exhibited the molecular ion at $m/e$ 550.4171 corresponding to $C_{40}H_{54}O$ (calcd. 550.4174), thus confirming a dehydrogenation of the $\beta,\beta$-caroten-2-ol under acidic conditions. Fragment ions were observed at $m/e$ 548 (M-2), 458 (M-92), 444 (M-106), and 392 (M-158). In contrast to the 2-ol the elimination of xylene was strongly favoured over the loss of toluene (M-92/M-106 = 0.75) in the acid-product. The intensity ratio of these two fragment ions is

known to be a function of the number of double bonds in the conjugated chain of the carotenoid [25]. The value of 0.75 fits very well with the existence of ten in-chain double bonds in the acid-product under investigation. This is a mass spectrometrical evidence for its *retro* structure. Since eleven conjugated double bonds are postulated from the electronic spectrum one of them must be located in one of the end rings. Peaks at $m/e$ 385 (M-165) and 345 (M-205) resulting from elimination of a $C_{11}H_{17}O$ and $C_{14}H_{21}O$ fragment, respectively, as established by high precision measurements, were attributed to cleavages of the 8,9 and 10,11 bonds with hydrogen transfer to the smaller fragments. Similarly, ions at $m/e$ 427 (M-123), 401 (M-149), and 361 (M-189) were due to rupture of the 6′,7′ bond (loss of $C_9H_{15}$), 8′,9′ bond (loss of $C_{11}H_{17}$), and 10′,11′ bond (loss of $C_{14}H_{21}$), respectively. Consequently, the endocyclic double bond was located in the $\beta'$-end group not carrying the oxygen function. Interestingly, no rupture of the 6,7-bond was observed in the high mass region. However, fragments at $m/e$ 139 ($C_9H_{15}O$) and 137 ($C_9H_{13}O$) were associated with this cleavage due to charge retention by the smaller fragment with hydrogen transfer once to the smaller and once to the larger fragment. Fragmentations in analogy to this one were observed for other cleavages. Generally, those ions were favoured which were associated with a) ruptures nearby and end group, b) ruptures nearby the non-oxygenated end group, c) hydrogen transfer to the uncharged larger fragment. Conclusively, the structure of the product obtainied by acid treatment of $\beta,\beta$-caroten-2-ol was assigned to 4′,5-*retro*-$\beta,\beta$-caroten-2-one, which is in full agreement with all data observed.

*Retro* carotenoids are strongly adsorbed on polyamide layers [10]. As shown in Fig. 8 quite similar to the dehydration product of the 4-ol the product under investigation did not migrate in the solvent used in contrast to the untreated 2-ol. This is a chromatographical support for the *retro* structure.

Since the hydroxyl group had been changed to a carbonyl group the *retro* product yielded no acetate; however, all attempts to reduce the ketone again by NaBH₄ or LiAlH₄ failed. This is surprising, and may be a result of shielding by the neighbouring dimethyl group.

Some experiments were carried out on the conditions of the acid catalyzed dehydrogenation and
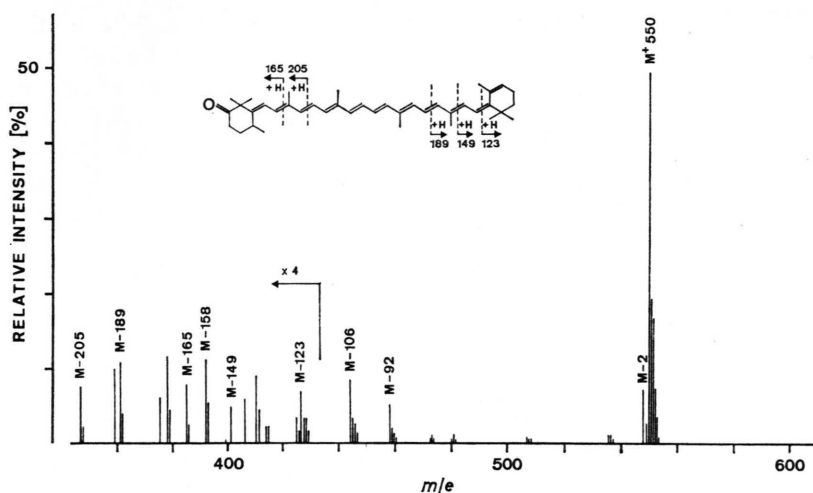
Fig. 9. Mass spectrum (70 eV; 8 kV; 170 °C) of the *retro* product obtained by acid treatment of β,β-caroten-2-ol. x 4 — peaks are magnified by factor 4.

*retro* rearrangement of β,β-caroten-2-ol. This reaction proceeded not only with BF₃ as a Lewis acid but also with HCl. Washing the commercial chloroform ethanol-free was without any effect on the reaction. As already mentioned, the same product is equally formed in ethanol solution but markedly slower. When studying the time course of this dehydrogenation in pure oxygen and under nitrogen a considerable enhancement of the reaction by oxygen was established (Fig. 10). Prolonged treat-



Fig. 10. Time courses of dehydrogenation of β,β-caroten-2-ol (▲) to the *retro* product (●) by 0.1 M BF₃ in chloroform under nitrogen and oxygen, respectively.

ment in oxygen, however, caused a marked loss of the *retro* product while a polar compound was accumulated which absorbed at (424), 446, and 474 nm in acetone (% II/II = 45). Since no spectral change was observed upon addition of HCl the presence of a 5,6-epoxy group[24] was excluded. Ac-

cording to its chromatographic properties it could be a diol. This pigment was not investigated further. Optimal yields of the *retro* product were obtained after a 30 min treatment in air. The results indicate that molecular oxygen may be the acceptor for the hydrogen eliminated from the secondary alcohol group of the 2-ol. The still proceeding reaction under nitrogen may be explained by traces of oxygen which could be quite effective in the microgram quantities of carotenoids used.
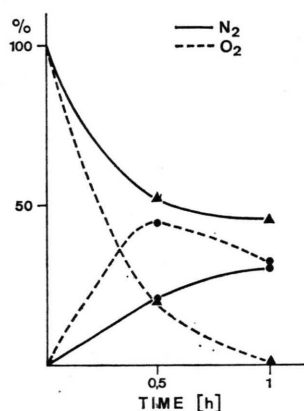
## On the biosynthesis of β,β-caroten-2-ol

Since animals are considered to lack the ability for a *de novo* synthesis of carotenoids[26] all carotenoids, which are found in an animal but not in its food must be derived from dietary carotenoids. Accordingly, the β,β-caroten-2-ol present in relative large amounts in *Cerura* should be synthesized from β,β-carotene of plant origin. In the algal 2-ols a formation via 1,2-epoxides in connection with the cyclization of the carotenoids has been considered[3]. Since β,β-carotene is already a bicyclic precursor the mechanism of introduction of a 2-ol group should be different in insects. But the intermediate formation of 1,2-epoxides from β,β-carotene can not be discarded until precise information is obtained on the mechanism of this hydroxylation in insects.

## Analytical remarks

Following a critical discussion of Lee and Gilchrist[4] 2-hydroxylated carotenoids have not been

detected during earlier studies in this field due to insufficient analytical tools and careless interpretations of results. In accordance with Kjøsen *et al.*[3] spectroscopic evidence on 2-hydroxy carotenoids is only obtained from NMR measurements requiring relative large quantities. In micro-scale work the lower polarity of 2-hydroxylated carotenoids relative to the other isomers is a first hint at these structures. In the TLC systems reported so far[3, 4] β,β-caroten-2-ol is well separated from the corresponding 3-ol and 4-ol, the latter both, however, are not or hardly distinguished. The same is true for the adsorption system used in the present work. In contrast, the silica gel-G system separates the 2-ol and 4-ol from the more polar 3-ol. Therefore, successive chromatography of a mono-ol fraction in the silica gel-G and adsorption system will provide all three isomeric mono-ols free of each other (*cf* Fig. 3A, B). As demonstrated in the reversed-phase system true partition chromatography is the only way of an unequivocal identification of β,β-caroten-2-ol and the corresponding 3-ol and 4-ol during a single run (*cf* Fig. 3C).

Regarding the chemical behaviour of hydroxylated carotenoids, generally, attention is drawn to the diagnostic value of acetylation cinetics which are influenced by both character and vicinity of the hydroxyl groups. In the 2-hydroxylated carotenoids negative results of etherification and of allylic oxidation tests have been the sole basis for a chemical discrimination from the related 4-ols so far[4]. The 3-ols, however, show also no reaction. Now, the dehydrogenation and rearrangement to a *retro* chromophore reported for β,β-caroten-2-ol seems to be specific to this substitution type[5, 7], and therefore, this reaction is of diagnostic value when only minute amounts of pigments are available. Furthermore, this chemical test may be carried out also on mixtures of β,β-caroten-2-ol and β,β-caroten-4-ol since their *retro* products obtained by acidchloroform treatment are easily separated by chromatography due to their different polarities, and exhibit different electronic spectra. Therefore, product control should be done in any experiment.

[1]  D. Bückmann, Biol. Zbl. **72**, 276 [1953].
[2]  H. Nagel, Thesis, University Giessen, Giessen 1970.
[3]  H. Kjøsen, N. Arpin, and S. Liaaen-Jensen, Acta Chem. Scand. **26**, 3053 [1972].
[4]  W. L. Lee and B. M. Gilchrist, Comp. Biochem. Physiol. **51 B**, 247 [1975].
[5]  H. Kayser, in preparation.
[6]  H. Kayser, in preparation.
[7]  H. Kayser, Tetrahedron Lett. **43**, 3743 [1975].
[8]  H. Kayser, Z. Naturforsch. **30 c**, 369 [1975].
[9]  A. Hager and T. Meyer-Bertenrath, Planta **69**, 198 [1966].
[10]  K. Egger and H. Voigt, Z. Pflanzenphysiol. **53**, 64 [1965].
[11]  H. Kayser, J. Comp. Physiol. **104**, 27 [1975].
[12]  H. Kayser, J. Insect Physiol. **20**, 89 [1974].
[13]  A. Hager and H. Stransky, Arch. Mikrobiol. **71**, 132 [1970].
[14]  Organikum, p. 459, Deutscher Verlag der Wissenschaften, Berlin 1964.
[15]  F. J. Petracek and L. Zechmeister, J. Amer. Chem. Soc. **78**, 3188 [1956].

[16]  L. Cholnoky, K. Györgyfy, E. Nagy, and M. Pánczél, Nature **178**, 410 [1956].
[17]  Appendix. In *Carotenoids* (O. Isler, ed.), Birkhäuser, Basel 1971.
[18]  S. Liaaen-Jensen, Kgl. Norske Videnskab. Seleskabs, Skrifter Nr. 8.
[19]  C. R. Enzell, G. W. Francis, and S. Liaaen-Jensen, Acta Chem. Scand. **23**, 727 [1969].
[20]  H. Kjøsen, S. Liaaen-Jensen, and C. R. Enzell, Acta Chem. Scand. **25**, 85 [1971].
[21]  J. E. Johansen, Å. Eidem, and S. Liaaen-Jensen, Acta Chem. Scand. **B 28**, 385 [1974].
[22]  G. W. Francis, Acta Chem. Scand. **26**, 1443 [1972].
[23]  W. Vetter, G. Englert, N. Rigassi, and U. Schwieter, In *Carotenoids* (O. Isler, ed.), Chapter IV, Birkhäuser, Basel 1971.
[24]  G. Nybraaten and S. Liaaen-Jensen, Acta Chem. Scand. **B 28**, 485 [1974].
[25]  C. R. Enzell, G. W. Francis, and S. Liaaen-Jensen, Acta Chem. Scand. **22**, 1054 [1968].
[26]  B. L. C. Weedon, In *Carotenoids* (O. Isler, ed.), Chapter II, Birkhäuser, Basel 1971.