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CAROTENOIDS OF HIGHER PLANTS—I

THE STRUCTURES OF LYCOXANTHIN AND LYCOPHYLL

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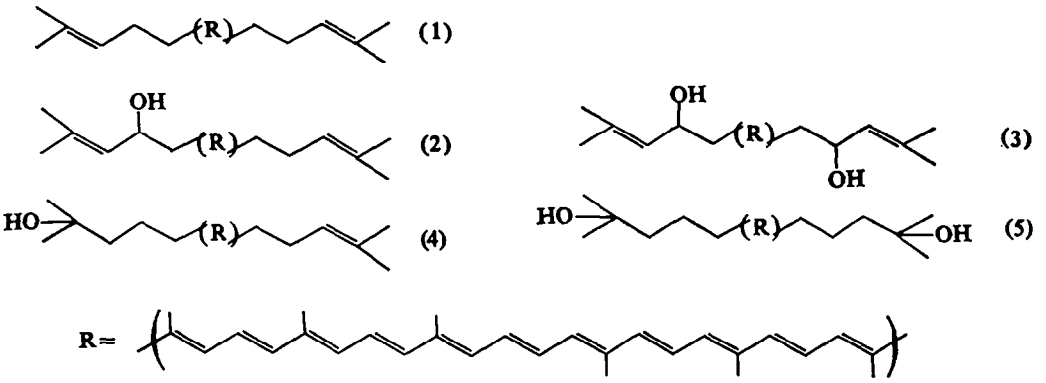
Abstract—The structures of the carotenoids lycoxanthin and lycophyll, previously recognized as lycopen-3-ol (2) and lycopene-3,3′-diol (3) respectively, are shown to be primary alcohols; lycoxanthin being lycopen-16-ol (6) and lycophyll lycopene-16,16′-diol (9).

INTRODUCTION

THE carotenoids of the red berries of *Solanum dulcamara* were first investigated by Zechmeister and Cholnoky.[1] In addition to lycopene (1), a mono-ol ($C_{40}H_{56}O$) and a diol ($C_{40}H_{56}O_2$) with similar visible spectra to (1) were isolated. The mono-ol, lycoxanthin, was characterized by the formation of a monoacetate, and the diol, lycophyll, by the formation of a dipalmitate. Proof for the position of the hydroxyl groups was not given and they were, by analogy with many cyclic xanthophylls, assumed to be located in 3,3′-position. The suggested structures for lycoxanthin (2) and lycophyll (3) have since been extensively quoted in the literature.

The presence of lycoxanthin has been claimed in various fruits[1–8] and berries,[1,9] as well



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as in fungi,[10,11] photosynthetic[12–16] and non-photosynthetic bacteria.[17] Lycophyll has also been regarded as a constituent of various berries[1,8] and micro-organisms.[13,15,16]

The pigment identified as lycoxanthin in photosynthetic bacteria[12–16] was later shown to be the tertiary alcohol rhodopin (4).[18] The presence of lycoxanthin in *Corynebacterium poinsettiae* has also subsequently been disproved.[19] The proof of identity of lycoxanthin in other sources is, in most cases, unsatisfactory, and its natural distribution is therefore uncertain. The diol from photosynthetic bacteria, identified as lycophyll,[13,15] was shown in one case to be 1,2,1′,2′-tetrahydro-lycopene-1,1′-diol (5)[20] and this may be true for other cases too.

In recent years lycoxanthin has been re-isolated from *S. dulcamara*.[21] Lack of allylic dehydration with acidified chloroform[22] was considered to support the structure (2) suggested for lycoxanthin, although the observed OH deformation or C—O stretching absorption at 1005 cm^{-1} appeared low.

In the present work a re-examination of the structures of lycoxanthin and lycophyll has been carried out by modern methods. They are shown to be (6) and (9) respectively.

TABLE 1. ADSORPTIVE PROPERTIES OF LYCOXANTHIN (6), LYCOPHYLL (9) AND VARIOUS DERIVATIVES THEREOF

Carotenoid	Required eluent from alumina activity grade 2	R_f-value on Schleicher & Schüll No. 287 paper	
		5% acetone*	10% acetone*
Lycopene (1)	5–15% ether*	0·98	
Lycoxanthin acetate (7)	20% ether	0·70	
Lycophyll diacetate (10)	50% ether		0·78
Lycopen-16-al (8)	50% ether	0·45	
Lycoxanthin (6)	100% ether	0·30	
Lycophyll monoacetate			0·45
Lycophyll (9)	2% methanol in ether		0·15

* In light petroleum.

RESULTS AND DISCUSSION

Pure lycoxanthin (6), m.p. 170°, was readily obtained from ripe berries of *Solanum dulcamara*. The visible (Fig. 1) and i.r. (Fig. 2) spectra corresponded to those previously

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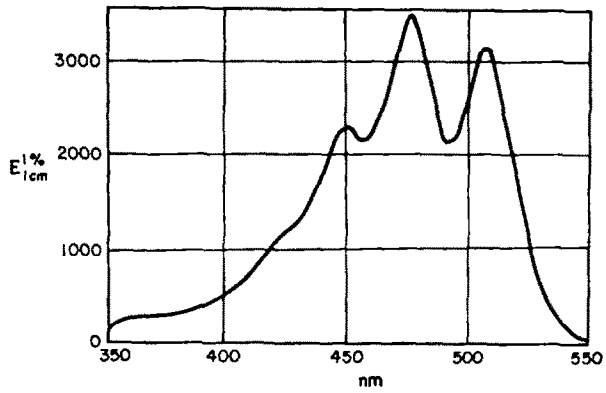


FIG. 1. ABSORPTION SPECTRUM IN VISIBLE LIGHT FOR LYCOXANTHIN (6) RECORDED IN ACETONE SOLUTION.

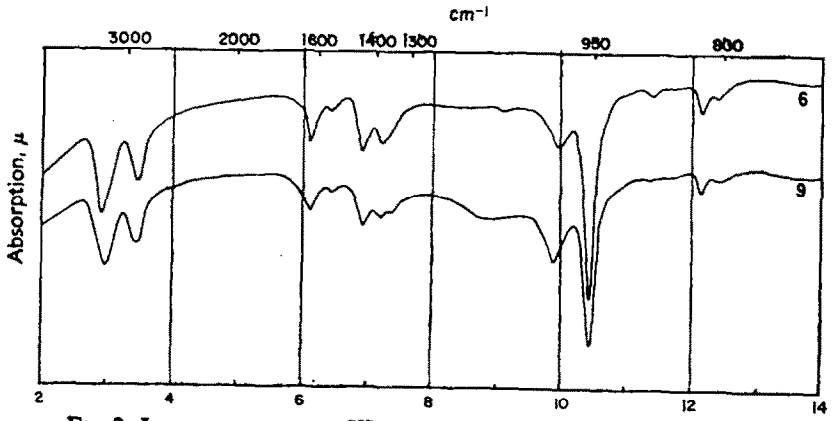


FIG. 2. INFRARED SPECTRA IN KBr OF LYCOXANTHIN (6) AND LYCOPHYLL (9).

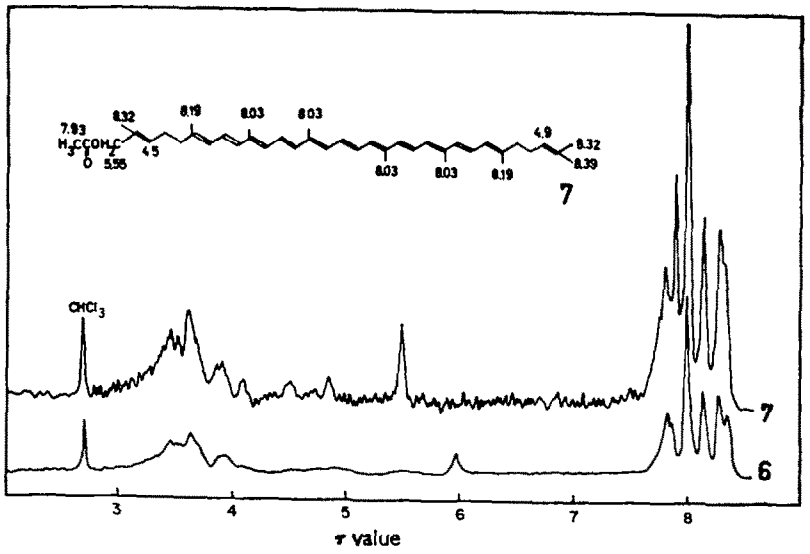
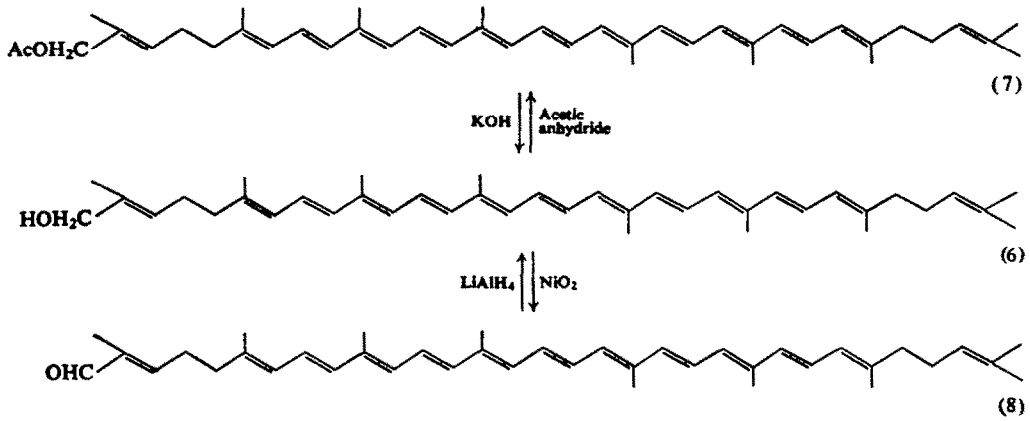


FIG. 3. NMR SPECTRA IN DEUTEROCHLOROFORM OF LYCOXANTHIN (6) AND LYCOXANTHIN ACETATE (7).

reported.[1,21] Due to the solubility properties in deuterochloroform, the proton magnetic resonance spectrum of the monoacetate (7) was most informative; see Fig. 3 including signal assignments.



The presence of four in-chain (8·03τ) and two end-of-chain (8·19τ) methyl groups in lycoxanthin (6) and lycoxanthin acetate (7) supports a location of the eleven carbon–carbon double bond chromophore identical to that of lycopene (1). The signals at 8·39τ (three protons) and 8·32τ (six protons) were interpreted as due to one *iso*propylidene end-group[23] and one hydroxylated end-group as in (6). The methylene of the hydroxymethyl group occurred as a singlet at 5·98τ (two protons) in lycoxanthin (6), shifted to 5·52τ in the acetate (7). The presence of four allylic methylene groups in the acetate (7) could be accounted for by the signals in the 7·8–7·9τ region in similar positions as reported for lycopene (1).[24] Furthermore the signals caused by the sixteen olefinic protons of the polyene chain were found in the 3·3–4·2τ multiplet. Finally the olefinic proton of the *iso*propylidene in lycoxanthin acetate (7) occurred at *ca.* 4·8τ in the same position as found for lycopene (1), whereas the olefinic proton of the second acetylated end-group in 7 appeared at lower fields (*ca.* 4·5τ).

Allylic oxidation with nickel peroxide[25,26] provided a monoaldehyde (8) with unchanged spectral properties in visible light (*cf.* Fig. 1). The aldehyde, lycopen-16-al (8) was not enolized by alkali, and was reduced to lycoxanthin (6) on treatment with lithium aluminium hydride.

Mass spectra of lycoxanthin (6, M = 552) and its aldehydic oxidation product (8, M = 550) supported the structures suggested and will be discussed elsewhere.[27]

It has been reported[21] that iodine catalysed isomerization of *trans* lycoxanthin (6) provided a stereoisomer (neo A) with unchanged spectral properties (identical fine structure in the visible light spectrum and lack of *cis*-peak). This isomer can now be conceived as the one with opposite configuration at the double bond carrying the hydroxymethyl group. The actual configuration of the hydroxylated double bond in (6) is not established.

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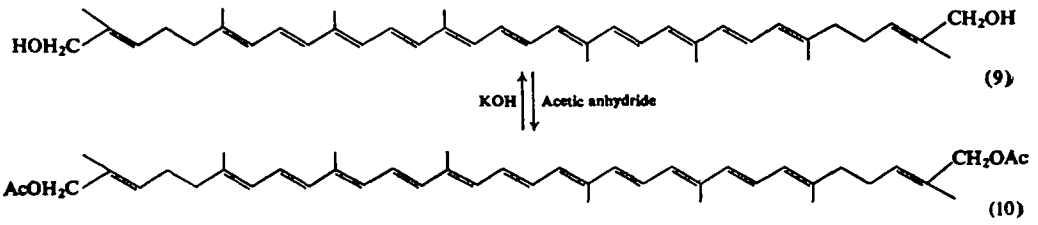
Lycophyll (9) occurred in lower quantity and was less easily purified. The i.r. spectrum (Fig. 2) closely resembled that of lycoxanthin (6), except that the relative intensity of the 1005 cm^{-1} absorption (primary hydroxyl) was much greater for lycophyll (9). Its molecular weight (M = 568) was secured by mass spectrometry,[27] and the presence of two primary or secondary hydroxyl groups accessible for acetylation[1] was confirmed. The primary character of these groupings, located in end-groups identical with the hydroxylated one of lycoxanthin (6), was revealed by the proton magnetic resonance spectrum of lycophyll diacetate (10), although the sample examined contained a non-carotenoid contaminant. The intensity of the 5·52τ signal (methylene in acetoxymethyl) and the 8·03τ signal (in-chain-methyl) was in the required ratio 1:3 for (10), as was the intensity ratio (1:2) of the 8·31τ (methyl on isolated double bond) and the 8·03τ signals. Moreover, the 8·39τ signal characteristic of the *iso*-propylidene end-group[23,24] was missing. Finally the fractionation pattern observed by mass spectrometry of lycophyll[27] is taken to support structure (9) for this xanthophyll. Again, the configuration around the double bond of the two end-groups is not established.



Carotenoids with primary hydroxyl groups have been considered rare in nature and have so far only been encountered in micro-organisms.[28,29] The finding of such primary carotenols also in higher plants indicates a more general distribution and calls for further caution in accepting xanthophyll structures proposed on the basis of insufficient evidence.

EXPERIMENTAL

Materials and Methods

Ripe berries (2·03 kg) of *Solanum dulcamara*, collected at Kjeller in October and stored in the deep-freeze, were used.

When not otherwise stated, the methods used were as previously described.[30] Column chromatography was carried out on Spence alumina, activity grade 2, and paper chromatography on paper with kieselguhr filler. Adsorptive properties are compiled in Table 1. Chemical reactions were followed by paper chromatography. The silylation procedure used has been described elsewhere.[31] NMR spectra were recorded in deutero-chloroform with tetramethylsilane as internal standard on a Varian HA 60 (60 Mc/sec) instrument and mass spectra on an LKB mass spectrometer with direct inlet system at conditions specified elsewhere.[27]

Isolation of the Carotenoids

The berries were allowed to thaw, and the carotenoids were most efficiently extracted with acetone in a Waring blendor. Addition of dry ice provided cooling and a protective atmosphere. The combined acetone extracts were treated as described elsewhere.[21] After column chromatography, lycopene ((1), 165 mg), lycoxanthin ((6), 100 mg) and lycophyll ((9), 10 mg) were isolated; estimated carotenoid content in fresh berries 0·016 per cent, previously reported 0·018 per cent.[21] Lycoxanthin (6) and lycophyll (9) comprised 38 and 4 per cent respectively of the total carotenoid; previously found 14 and 2 per cent.[21]

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Lycoxanthin (6)

Trans (6) crystallized as needles from ether–light petroleum, yield *ca.* 17 mg, m.p. 170°, previously reported 168°.[1,21] The absorption spectrum in visible light recorded in acetone (Fig. 1) had abs. max. at 448, 476 ($E^{1\%}_{1cm}$ = 3380–3500 for various aliquots) and 508 nm. The i.r. spectrum in KBr (Fig. 2) had abs. max. at 3400, 1380, 1360 (*CH*$_3$, *gem.* CH_3); 1005 (*prim.* allylic OH); 960 (*trans* disubst. double bonds) and 825 (*trans* trisubst. double bonds) cm^{-1}. The NMR spectrum (Fig. 3) had signals at τ 8·32, 8·39 (8·9 H); 8·19 (6·0 H); 8·03 (12·4 H); 7·85 (8·3 H); 5·98 (1·9 H); 3·2–4·9 (18·3 H). The mass spectrum had prominent peaks at m/e = 552 (M), 550 (M-2), 548 (M-4), 536 (M-16), 534 (M-18), 532 (M-20), 483 (M-69), 481 (M-71), 467 (M-85), 466 (M-86), 465 (M-87), 460 (M-92, toluene[24]), 446 (M-106, xylene[24]), 444 (M-108), 442 (M-110), 430 (M-122), 428 (M-124) and 426 (M-126). In light petroleum/95 per cent methanol (6) had partition ratio 72:28.

Lycoxanthin Acetate (7)

(6) (*ca.* 10 mg) was quantitatively acetylated in the usual manner. (7) was purified by column chromatography and crystallized from ether–light petroleum; yield *ca.* 6 mg, m.p. 145°, previously reported 137°.[1] The absorption spectrum in visible light recorded in acetone corresponded to that of (6); $E^{1\%}_{1cm}$ = 3320 at 476 nm. The i.r. spectrum in KBr had abs. max. at 3480 (overtone C=O); 2920 (CH); 1740 (acetate); 1620 (conj. double bonds); 1430 (CH_2), 1360 (CH_3 *gem.* CH_3); 1225 (acetate); 1020 (acetate) and 955 (*trans* disubst. double bonds) cm^{-1}. The NMR spectrum (Fig. 2) had signals at τ 8·39, 8·32 (8·8 H); 8·19 (6·1 H); 8·03 (12·0 H); 7·93, 7·85 (11·5 H); 5·55 (2·3 H), *ca.* 4·8 (1 H), *ca.* 4·5 (1 H) and 3·3–4·2 (*ca.* 16 H). The NMR spectrum of lycopene (1) had olefinic signals at τ *ca.* 4·8 (2 H) and 3·3–4·2 (16 H). Lycoxanthin acetate (7) could not be silylated and was hydrolysed to lycoxanthin (6) under standard saponification conditions.

Lycopen-16-al (8)

The reagents were tested with lutein which gave 3′-keto-α-caroten-3-ol in satisfactory yield. (6) (5 mg) in benzene (8 ml) was treated with NiO_2 (50 mg, available oxygen $4·6 \times 10^{-3}$ g atom/g NiO_2 determined by titration) for 40 min; pigment recovery was 46 per cent. The reaction mixture contained (8) (15 per cent) and unreacted (6) (80 per cent). The aldehyde (8), purified by column chromatography, exhibited the same absorption spectrum in visible light as (6) and had partition ratio 90:10 in light petroleum/95 per cent methanol. Spectral and partition behaviour were unchanged in the presence of alkali. (8) could not be acetylated or silylated and was reduced with $LiAlH_4$ in dry ether to (6). The mass spectrum of a concentrate had prominent peaks at m/e = 550 (M), 548 (M-2), 535 (M-15), 532 (M-18), 523 (M-27), 509 (M-41), 495 (M-55), 481 (M-69), 467 (M-83), 458 (M-92) and 444 (M-106).

Lycophyll (9)

Crystallized as needles from ether–light petroleum; yield 3·8 mg, m.p. 189°, previously reported 179°.[1] The absorption spectrum in visible light corresponded to that of (6) (see Fig. 1), $E^{1\%}_{1cm} \geq 3240$ at 508 nm in acetone. The i.r. spectrum (Fig. 2) had abs. max. in the same position as those given for lycoxanthin (6) above. The mass spectrum had prominent peaks at m/e = 568 (M), 566 (M-2), 564 (M-4), 552 (M-16), 550 (M-18), 548 (M-20), 534 (M-34), 532 (M-36), 483 (M-85), 476 (M-92) and 462 (M-106). Partition ratio in light petroleum/95 per cent methanol was 83:17.

Lycophyll Diacetate (10)

(9) (2·6 mg) was quantitatively acetylated in the usual manner. After column chromatography the eluates containing (10) were concentrated to dryness. A non-carotenoid contaminant was evident from its NMR spectrum and could not be removed by re-chromatography twice. The following signals of the NMR spectrum were ascribed to the carotenoid: τ 8·32; 8·19; 8·03; 7·93; 7·85; 5·55 and 3·3–4·2 with intensity ratios 6:6:31:4·9:18. The 7·93τ signal (acetate CH_3) apparently had some contribution from a non-carotenoid contaminent. No signals were present at 8·39τ.

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