

Carotenoids of Higher Plants

4. * The Stereochemistry of Lycopanthin and Lycophyll

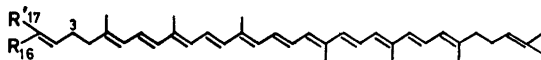
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The stereochemistry of the hydroxy-substituted isopropylidene group of lycopanthin has been examined by PMR spectroscopy together with isomerization studies and synthesis of model compounds. It is concluded that lycopanthin is 16-hydroxy-lycopene (1) with *trans* configuration between the hydroxymethyl group and the main chain of carbon atoms. The previously described neo A isomer, obtained on iodine catalyzed isomerization, has *cis* configuration around this terminal double bond.

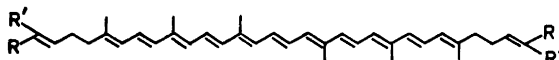
It is inferred that lycophyll is 16, 16'-dihydroxy-lycopene (3).

Lycopanthin, a characteristic carotenoid component in ripe berries of *Solanum dulcamara*, was for a long time considered to be 3-hydroxy-lycopene.¹ Recently it was independently demonstrated by two teams^{2,3} that the hydroxy group is primary, and present in a hydroxylated isopropylidene end group. Lycophyll was shown to be the corresponding diol.^{2,3} However,



1 R = CH₂OH, R' = CH₃

2 R = CH₃, R' = CH₂OH



3 R = CH₂OH, R' = CH₃

4 R = CH₃, R' = CH₂OH

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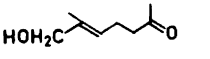
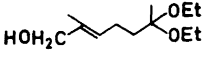
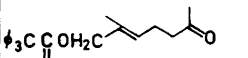
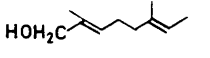
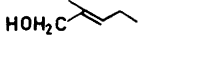
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it was not established whether lycoxanthin was 16- or 17-hydroxy-lycopenene (1 or 2), nor could the corresponding differentiation for lycophyll (3 or 4) be made.

RESULTS AND DISCUSSION

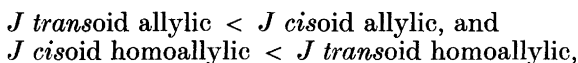
Pure, crystalline lycoxanthin was re-isolated from *Solanum dulcamara*. All-*trans* configuration for the polyene chain in lycoxanthin is revealed by its electronic spectrum, identical with that of all-*trans* lycopenene.⁴ The PMR-spectrum agreed with that previously reported.² Particular attention was paid to the signal position and line widths at half height (W_H) of the CH_2OH resonance (Table 1). Data for compounds with analogous end groups indicate that the methylene signal of the *cis*-ol occurs at lower field than that of the corresponding *trans*-ol⁵ (see Table 1, compounds 11, 12). However, the difference is small, and the chemical shift obviously influenced by other structural elements. It is therefore not possible to differentiate between *cis* and *trans* configuration on the basis of only the one hydroxy isomer. In the corresponding aldehyde series, however, such differentiation is possible on the

Table 1. PMR data for some compounds related to lycoxanthin (1).

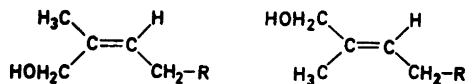
Compound	τ -value CH_2OR			W_H in Hz		Solvent	Ref.
	<i>trans</i>	<i>cis</i>	Δ <i>trans-cis</i>	<i>trans</i>	<i>cis</i>		
Lycoxanthin (1), natural	6.00			ca. 3		$CDCl_3$	
isomerized	6.00	5.86	0.14	ca. 3	ca. 2	$CDCl_3$	
 (9)	6.16	6.02	0.14	ca. 3	ca. 2	CCl_4	
 (8)	6.14	6.00	0.14	ca. 3	ca. 2	$CDCl_3$	
 (10)	5.54	5.39	0.15	3.0	2.4	CCl_4	
 (11)	6.16	6.02	0.14				5
 (12)	6.08	5.92	0.14			$CDCl_3$	5
Lycoxanthin (1) acetate	5.55			ca. 3		$CDCl_3$	2
Lycophyll (3) acetate	5.55			ca. 3		$CDCl_3$	2

basis of chemical shifts alone.^{5,6} Allylic oxidation to the corresponding aldehyde was carried out with silver carbonate-celite⁷ with somewhat improved yield, relative to the previously used method with nickel peroxide.^{8,2} but still in a yield unsatisfactory for PMR analysis. Under the same conditions, geraniol and lutein gave citral and 3-hydroxy- β,ϵ -caroten-3'-one (by new nomenclature⁹) in high yield.

It had previously been observed⁴ that all-*trans* lycoxanthin on iodine catalyzed stereoisomerization provided a neo A isomer (20 % of the equilibrium mixture) with electronic spectrum identical with that of the *trans* isomer. This phenomenon was not readily explicable on the basis of the old lycoxanthin structure, but may be explained from structures 1, 2 as isomerization around the 1 double bond. However, an alternative interpretation that also had to be considered was isomerization of the 5 or 5' double bonds. Such isomerism, causing no changes in the electronic spectrum, has hitherto only been demonstrated for gazaniaxanthin-rubixanthin.¹⁰ In the present work, the neo A isomer comprised 22 % of the isomerization mixture, in good agreement with previous results.⁴ The PMR-spectrum of the isomerization mixture exhibited an extra signal at τ 5.84, integrating for 24 % of the main methylene signal at τ 6.00. For the latter signal, $W_H = ca.$ 3 Hz, corresponding to the half band width measured for natural, unisomerized lycoxanthin, and of the former band, $W_H = ca.$ 2 Hz. The signal from the methylene group in such a *cis* allylic alcohol is expected to occur at lower field⁵ and to be narrower than the *trans* signal, since the methylene protons are *transoid* allylically coupled (to one proton) and *cisoid* homo-allylically coupled (to two protons), because normally:^{11,12}



although exceptions are reported.



cis: transoid allylic
cisoid homoallylic

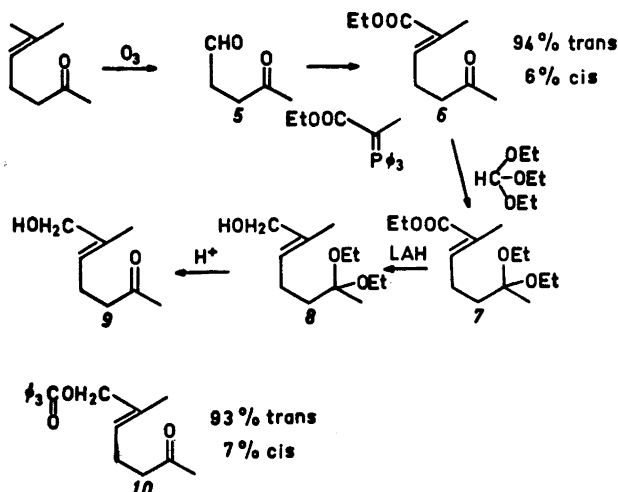
trans: cisoid allylic
transoid homoallylic

The results consequently indicated (a) that natural lycoxanthin exhibited *trans* configuration (*I*) between the largest substituents of the hydroxylated isopropylidene group, and (b) that the neo A isomer exhibited *cis* configuration around the same double bond.

The interpretation of the above PMR evidence was confirmed by examination of some model compounds prepared as part of our synthetic programme, Scheme 1. All products had satisfactory spectral (IR, PMR, and mass spectra) properties.

The Wittig reaction of levulaldehyde (*5*) with methyl carbethoxytriphenylphosphorane was found to be specific for the aldehyde group and to give predominantly the *trans* product (*6*), as expected.¹³ The presence of *cis* product

was readily shown by the PMR-spectrum, the olefinic proton signal being found in the expected position¹⁴ upfield of that in the *trans* compound, but the amount ($6\% \pm 2\%$ by integration) was difficult to determine because of the broadness and relative weakness of the olefinic proton signal. The keto



ester (6) was converted to the ketal (7). Reduction of the ester group gave the corresponding primary allylic alcohol (8) with "lycoxanthin end-group", whose PMR-spectrum showed a broad signal at τ 6.15, and a smaller signal at τ 6.01, attributed to CH_2OH in the *trans* and *cis* isomer. Determination of the relative amounts of the isomers and assignment of *cis/trans* structure were made from the PMR-spectrum of the triphenyl acetate (10), where the relevant signals were rather sharper. The ketal alcohol (8) was converted to the keto alcohol (9) by standard acid hydrolysis, and the keto alcohol (9) was finally esterified to the triphenyl acetate (10). Repeated careful integration of the signals ascribed to CH_2OAcyl in the *trans* and *cis* isomer of 10 showed the smaller peak to be 6.7% of the total. Under identical experimental conditions (except amplitude, which had to be increased 10 times to measure the small signal), $W_{\text{H}} = 2.4$ Hz for the smaller signal, and $W_{\text{H}} = 3.0$ for the larger signal. The former was 8.5 Hz downfield, for the same reasons as discussed above, and was ascribed to the *cis* isomer. The result is as expected from reduction of an ester (6) containing 6% *cis* isomer.

Regarding the other W_{H} values given in Table 1, these are measured with less accuracy than for 10 and are considered approximate figures. However, assignments of the *cis* and *trans* signals (Table 1) are further supported by the constant $\Delta\tau$ value between these signals for each compound studied.

The PMR results for compounds 8, 9, 10, 11, and 12 are thus considered fully consistent with the conclusions reached above concerning the stereochemistry of the hydroxy-methyl substituted terminal double bond of lycoxanthin.

Since the chemical shift (τ 5.55) of the relevant methylene group of lycophyll diacetate coincides with that of lycoxanthin (*I*) acetate² (Table 1), it may be concluded that both hydroxymethyl groups of lycophyll are *trans* to the major substituent, and hence that lycophyll is 3. It may be mentioned that *trans* configuration has also been assigned to the analogous terminal double bonds in *dehydrogenans*-P439,^{15,6} now referred to as decaprenoxanthin.¹⁶ In the latter case, PMR data for the corresponding dialdehyde was considered conclusive evidence.⁵

Iodine catalyzed stereoisomerization of lycophyll (3) did not result in formation of chromatographically distinct isomers with *cis* configuration around the terminal double bonds, nor was this phenomenon encountered for lycopene-16-al here prepared, or for any of the C₅₀-carotenoids with related end-groups studied in our laboratory. This may be due to energetic reasons or to inadequate separation methods. The most abundant *cis* isomer (neo A) of lycophyll (3) appears, according to the general theory,¹⁷ to have one *cis* bond in the polyene chain near the end of the chromophore (9-position?), whereas the second *cis* isomer described for lycoxanthin (*I*), neo B, appears to have one *cis* bond in 9, 13, 13' or 9'-position, and the neo C isomer of lycoxanthin two *cis* bonds, one of which should be in a near-to-central position.

EXPERIMENTAL

General. Particulars concerning the isolation of crystalline carotenoids were as summarized elsewhere.¹⁸ Electronic spectra were recorded on a Coleman-Hitachi 124 spectrometer, IR spectra on a Perkin Elmer 257 Grating spectrometer, PMR spectra on a Varian A-60A instrument, and mass spectra on an MS902 spectrometer.

Lycoxanthin (*I*) and lycophyll (3)

Isolation of the carotenoids. Ripe berries (3.7 kg) of *Solanum dulcamara*, collected near Kjeller in September 1968 and October 1969, were deep-frozen and extracted by the general method;^{3,4} total amount of carotenoids 1.1 g, corresponding to 0.14 % of the acetone extracted residue. The crude carotenoid mixture was saponified, transferred to ether, and submitted to repeated partition between petroleum ether and 95 % aqueous methanol to effect separation of lycoxanthin and lycophyll from lycopene. The hypophasic pigments were subsequently chromatographed on deactivated alumina and crystallized; yield crystalline lycoxanthin, 18 mg, and crystalline lycophyll, 2 mg. Calculation of the content of various fractions showed that lycopene comprised ca. 88 %, lycoxanthin ca. 10 %, and lycophyll ca. 2 % of the total carotenoid.

Lycoxanthin (I), m.p. 171°C (evacuated tube), reported 168°C,¹ had λ_{\max} (423), 446, 475 [*E* (1 %, 1 cm) = 3420] and 505 nm in acetone, ν_{\max} (KBr) in agreement with reported spectra,^{2,4} τ (CDCl₃), 8.39 (1 Me), 8.32 (2 Me), 8.19 (2 Me), 8.00 (4 Me), 6.00 (2 H, W_H = ca. 3 Hz) and 3.4–4.9 (ca. 18 H) in agreement with previous spectra,² and *m/e* 552 (M), M–2, M–16, M–18, M–69, M–92, M–106 etc, as reported earlier.^{3,19}

Iodine catalyzed isomerization of *I* (4 mg) in benzene (100 ml) was followed paper-chromatographically and spectrophotometrically. The equilibrium mixture (λ_{\max} 370, 365, 482, and 515 nm in benzene), resolved on circular kieselguhr paper (5 % acetone in petroleum ether) comprised *trans* (45 % of total, R_F = 0.36, λ_{\max} in acetone 365, 448, 474, and 505 nm, % D_B/D_{II} = 10, % III/II = 58; neo A (22 %, R_F = 0.40, λ_{\max} 365, 448, 474, and 505 nm, % D_B/D_{II} = 11, % III/II = 53); neo B (20 %, R_F = 0.53, λ_{\max} 362, 443, 468, and 499 nm, % D_B/D_{II} = 28, % III/II = 25), and neo C (13 %, R_F = 0.69, λ_{\max} 362, 440, 466, and 497 nm, % D_B/D_{II} = 47, and % III/II = 16).

Allylic oxidation with Ag_2CO_3 -celite ⁷ of *I* (0.1–3.0 mg) at room temperature or 40°C could not be effected. *I* (7.6 mg) in dry benzene (8 ml), treated with Ag_2CO_3 -celite (236 g), gave 43% pigment recovery. The reaction mixture contained *cis*-isomerized lycopene-16-al (11%) and *trans*-lycopen-16-al (4%). *trans*-Lycopen-16-al had $R_F = 0.61$ on kieselguhr paper (5% acetone in petroleum ether), λ_{max} as *trans* lycoxanthin (*I*) characteristic, ν_{max} (KBr) 1680 (conj. aldehyde). Iodine catalyzed isomerization of *trans* lycopen-16-al gave a neo A isomer (38% of total, $R_F = 0.72$ on kieselguhr paper, using 5% acetone in petroleum ether, λ_{max} 363, 442, 468, and 499 nm, % $D_B/D_{II} = 13$, % $III/II = 45$) in addition to the *trans* isomer.

For comparison, geraniol (154 mg) treated with Ag_2CO_3 -celite (2.28 g) in dry benzene (60 ml) at 80°C for 1 h was quantitatively (PMR) converted to citral. Lutein (1.27 mg) in dry benzene (2 ml) treated with Ag_2CO_3 -celite (33 mg) at 40°C for 6 h gave 93% pigment recovery and no new products. Analogous treatment at 80°C for 1.5 h gave 62% pigment recovery. The desired 3'-keto product (*cf.* Ref. 20) comprised 40% of the recovered pigment.

Lycophyll (3), m.p. 191°C, reported 179°C,¹ had λ_{max} in acetone at (423), 446, 475 [$E(1\%, 1\text{ cm}) = 3200$] and 505 nm, λ_{max} (KBr) as for lycoxanthin with stronger absorption at 1010 cm^{-1} .

The equilibrium mixture (λ_{max} 370, 457, 481, and 513 nm in benzene) obtained on iodine catalyzed isomerization consisted of neo A (48% of total, $R_F = 0.62$ on kieselguhr paper with 15% acetone in petroleum ether, λ_{max} 361, 443, 468, and 499 nm in acetone % $D_B/D_{II} = 17$, and % $III/II = 30$) and *trans*-lycophyll (52%, $R_F = 0.48$).

Synthetic model compounds

Ethyl 6-keto-2-methylhept-2-enoate (6). A solution of levulaldehyde (5, 5 g, prepared by ozonolysis of 6-methyl-hept-5-en-2-one²¹ and used directly) in ethyl acetate was added to a solution of carbethoxytriphenylphosphorane²² (20 g) in ethyl acetate (total volume 100 ml) and refluxed for 20 h. Evaporation of the solvent and decolorising on a charcoal column gave 11.5 g of almost colourless oil, used directly in the next step.

A small sample distilled under vacuum (b.p. 80°C, 0.2 mmHg) had $n_D^{25} = 1.4389$. Characteristic spectral properties: m/e 184.110 (M), calc. for $\text{C}_{16}\text{H}_{16}\text{O}_3$, $M = 184.1093$; ν_{max} (liq.) 1720, 1710, 1650 cm^{-1} ; $\tau(\text{CDCl}_3)$ 3.4 multiplet (1 H), 6.89 quartet ($J = 7$ Hz, 2 H), 8.72 triplet ($J = 7$ Hz, 3 H), 8.2 (3H), 7.91 (singlet, 3 H), and no aldehyde signal, were in accordance with the structure assigned. The product contained about 6% *cis* isomer.

A dinitrophenylhydrazone was prepared by standard method, m.p. 121–123°C.

Ethyl 6,6-diethoxy-2-methylhept-2-enoate (7). The keto ester (6, 3 g), triethyl orthoformate (2.55 g), and ethanol (1.5 ml) were kept under nitrogen at 20°C overnight with a trace of *p*-toluene sulphonic acid. One drop of pyridine was then added, and the mixture poured into saturated sodium bicarbonate solution and extracted with ether. The ether layer was washed, dried (MgSO_4) and evaporated to dryness; yield 4 g (95%).

Characteristic spectral properties: m/e 243.1596 (M–15), calc. for $\text{C}_{13}\text{H}_{23}\text{O}_4$, 243.1596; 213.1491, (M–45), calc. for $\text{C}_{12}\text{H}_{21}\text{O}_3$, 213.1491; ν_{max} (liq.) 1710, 1650 cm^{-1} ; $\tau(\text{CDCl}_3)$ 3.35 multiplet (1 H), 5.90 quartet ($J = 7$ Hz, 2 H), 6.60 quartet ($J = 7$ Hz, 4 H), 8.20 doublet ($J = 1$ Hz, 3 H), and 8.6–9.0 two triplets, one singlet (total 12 H), were in accordance with the ketal structure.

6,6-Diethoxy-2-methylhept-2-en-1-ol (8). The ketal ester (7, 3.75 g) was dissolved in dry (LiAlH_4) ether (25 ml), and LiAlH_4 (300 mg, ca. 100% excess) was added in portions. The reaction mixture was stirred for 1 h under nitrogen at 20°C, a further 90 mg LiAlH_4 was added, and stirring continued for another 3 h. The product was isolated by adding an ice-cold saturated aqueous solution of ammonium chloride, until the insoluble material formed a solid mass with a clear supernatant. The ether layer was decanted, and the residue triturated with ether. The ether layers were combined and evaporated to constant weight; yield 2.79 g (89%).

Characteristic spectral properties: ν_{max} (liq.) 3400 cm^{-1} (broad) and $\tau(\text{CDCl}_3)$ 4.7 multiplet (1 H), 6.0 and 6.15 broad singlets (2 H), sharper on shaking with D_2O , 6.60

quartet ($J = 7$ Hz, 4 H), 8.38 broad singlet (ca. 3 H), 8.75–9.1 two triplets and one singlet (total 9 H), showed reduction of the ester group and retention of the double bond.

7-Hydroxy-6-methylhept-5-en-2-one (9). The ketal (8, 2.8 g) was dissolved in ether (20 ml), and water (20 ml) and concentrated hydrochloric acid (2 drops) added. The reaction mixture was kept under nitrogen at room temperature overnight with sufficient magnetic stirring to keep the two layers mixed. Sodium carbonate (200 mg) was added and the ether layer separated. Magnesium sulphate was added to the aqueous phase, which was then extracted with ether four times. The ether extracts were combined, dried and evaporated, yielding 1.65 g (90 %) of ketol, free of ketal.

Characteristic spectral properties; ν_{\max} (liq.) 1712 cm^{-1} ; τ (CDCl_3) 4.6–4.9 multiplet (1H), 6.18 (6.03) broad singlet (3H), 7.92 singlet (3H), 8.36 broad singlet (3H) were in agreement with structure 9.

7-Triphenylacetoxy-6-methylhept-5-en-2-one (10). The hydroxyketone (9, 750 mg, 5.28 mmol) was dissolved in dry pyridine (1 ml, 12.4 mmol) and dry benzene (2 ml), and a solution of triphenylacetyl chloride (2 g, 6.5 mmol) in dry benzene (5 ml) was added, and the reaction mixture allowed to stand for 80 h at 20°C, followed by 3 h at 80°C. The mixture was cooled, diluted with ether, and the ether phase washed with sodium carbonate, dilute acetic acid and water, then dried and evaporated. The residual oil partly crystallized (triphenylacetyl chloride). The non-crystalline residue was chromatographed on silica and eluted with chloroform; 760 mg (35 %) of the required ester (9) was obtained, free of starting material.

Characteristic spectral properties: τ_{\max} (liq.) 1725 (broad) and 1600 cm^{-1} ; τ (CDCl_3) 2.82 singlet (15 H), 4.80 multiplet (1 H), 5.35 and 5.51 broad singlets (2 H), 7.8 broad multiplet (4 H), 8.10 sharp singlet (3 H), and 8.60 broad singlet (3 H) were in accordance with the proposed structure. From the PMR spectrum, 6.7 % of the *cis* isomer was estimated.

A dinitrophenylhydrazone was prepared in the usual way, chromatographed ($\text{SiO}_2/\text{CHCl}_3$) and crystallized from ethanol-ethyl acetate, m.p. 128–131°C, *m/e* 592.2322 (M), calc. for $\text{C}_{34}\text{H}_{32}\text{N}_4\text{O}_6$ $M = 592.2320$.

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