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LACTUCAXANTHIN, AN ε,ε-CAROTENE-3,3′-DIOL FROM *LACTUCA SATIVA**†

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Key Word Index—*Lactuca sativa*; Compositae; new carotenoid; lactucaxanthin; (3*R*,6*R*,3′*R*,6′*R*)-ε,ε-carotene-3,3′-diol; chemosystematics.

Abstract—Chemical and spectroscopic evidence including ^{1}H NMR and CD is presented, demonstrating the (3*R*,6*R*,3′*R*,6′*R*)-ε,ε-carotene-3,3′-diol structure of a new carotenoid, lactucaxanthin. Lactucaxanthin, isolated from *Lactuca sativa*, is the sixth chiral isomer encountered in nature of the ten possible chiral isomers of ε,ε-carotene-3,3′-diol. In a chemosystematic screening, lactucaxanthin was restricted to *Lactuca* and a few closely related genera within the tribe Cichorieae of the Compositae.

INTRODUCTION

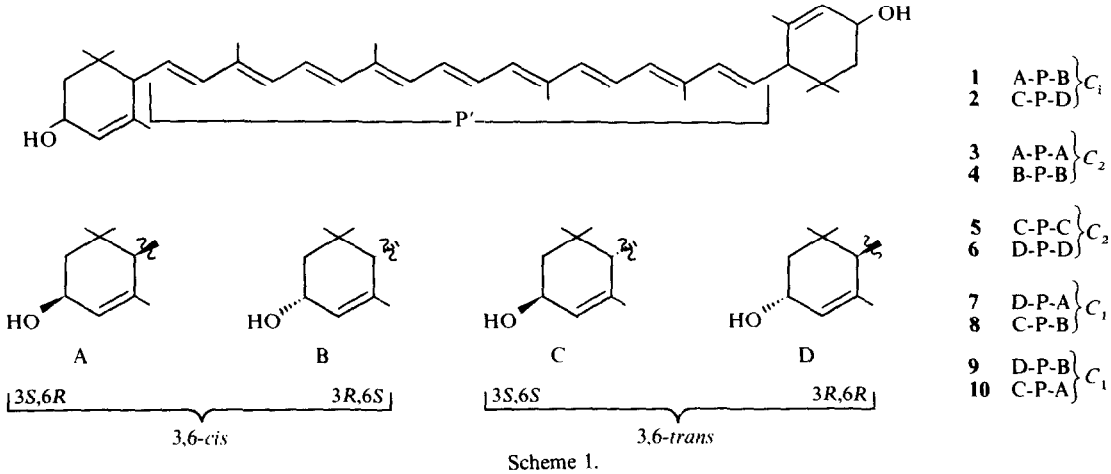
A total of ten chiral isomers with the ε,ε-carotene-3,3′-diol constitution are possible [1]. These include two optically inactive *meso* compounds with C_i symmetry (**1** and **2**, Scheme 1), two sets of enantiomers with C_2 symmetry (**3**, **4** and **5**, **6**) and two sets of enantiomers with C_1 symmetry (**7**, **8** and **9**, **10**). Hitherto identified in nature are the chiriquixanthins A (**7**) and B (**3**) from a frog [2] both with 6*R*,6′*R* chirality and three chiral isomers of tunaxanthin from various fishes, namely **8** [1, 3], **4** and **5** [1], all with 6*S*,6′*S* configuration. Moreover, a fourth isomer of tunaxanthin from *Sebastes flavidus* is considered to be a partly racemic chiriquixanthin B (**3**) [1].

We now report the isolation of the sixth chiral isomer of ε,ε-carotene-3,3′-diol, here called lactucaxanthin (**6**), isolated from chloroplasts of *Lactuca sativa* (green lettuce).

RESULTS AND DISCUSSION

Structural elucidation of stereoisomeric carotenoids of type **1**–**10** may now be carried out on a μg scale. The constitution follows from their electronic spectrum, revealing the nonaene chromophore [4], methylation of the hydroxy groups under conditions selective for allylic hydroxyl [5, 6] and mass spectroscopy [4]. Differentiation between 3,6-*cis* (A and B, Scheme 1) and 3,6-*trans* (C and D) end groups is readily made from the chemical shifts of the ^{1}H NMR signals of the methyl groups [1–3, 7]. The fact that the Cotton effect of substituted ε-rings is nearly exclusively determined by the chirality at C-6,6′ [7–10] allows unequivocal identification on the basis of combined ^{1}H NMR and CD evidence.

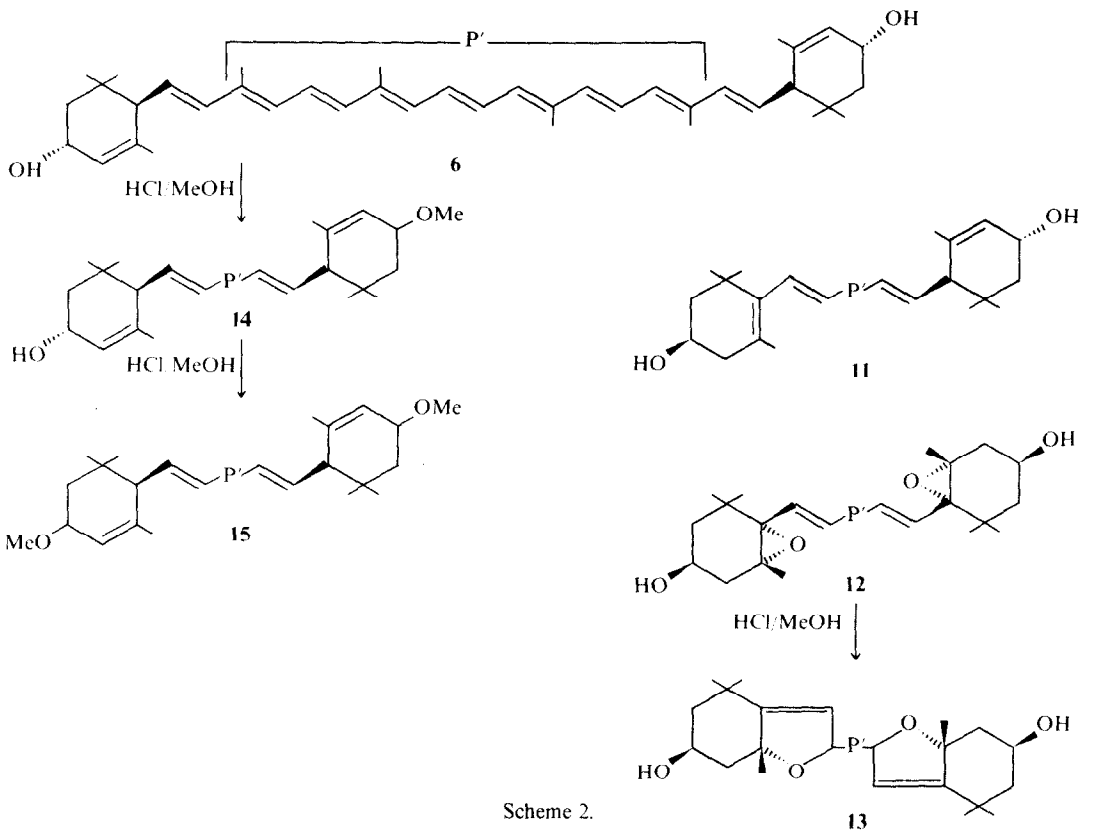
During an examination of the carotenoids of *L. sativa* in the German laboratory, a compound X, now called



Scheme 1.

* Dedicated to Professor C. H. Eugster on the occasion of his 60th birthday.

† Part 13 in the series "Carotenoids of Higher Plants". For Part 12 see (1979) *Phytochemistry* **18**, 303.



Scheme 2.

lactucaxanthin, with an electronic spectrum similar to violaxanthin (**12**, Scheme 2) was adsorbed between violaxanthin (**12**) and the carotenes on alkaline TLC plates [11]. On lipid-impregnated TLC plates [12], lactucaxanthin could not be separated from lutein (**11**).* Lactucaxanthin was not a 5,6-epoxide since it gave no products with shorter chromophore upon acid treatment under conditions where violaxanthin (**12**) underwent epoxide–furanoid rearrangement to auroxanthin (**13**) [13]. From these results, lactucaxanthin was suspected to be an ε,ε-carotene-diol.

Further studies of a fraction enriched in lactucaxanthin were carried out in Trondheim. Separation was achieved from lutein (**11**) on MgO plates [14] and by HPLC [15]. The electronic spectrum of lactucaxanthin with λ_{max} (acetone) 426,440 and 471 nm and pronounced spectral fine-structure (%III/II [16] = 93) confirmed the aliphatic nonaene chromophore. The MS with *m/e* 568 (M^+, consistent with $C_{40}H_{56}O_2$) and diagnostically useful fragment ions corresponding to the loss of one and two molecules of water, toluene and xylene, confirmed the carotenoid diol structure. The ^1H NMR ($CDCl_3$) spectrum showed methyl singlets at δ 0.85 (6 H), 1.00 (6 H) and 1.62 H (6 H) compatible with two *trans* 3-OH-ε rings, type C or D (Scheme 1) and two characteristic singlets for the four in-chain methyl groups. A two-proton multiplet centred at δ 4.2 accounted for the C-4,4′ olefinic protons.

Methylation of lactucaxanthin in 0.03 N acidic methanol provided an intermediary monomethyl ether (**14**) and a final dimethyl ether (**15**) with the predicted MS molecular and fragment ions.

The above evidence confirmed the ε,ε-carotene-3,3′-diol structure with two 3,6-*trans* configurated end groups. CD data with $\Delta\varepsilon$ (EPA) = +17.5 at 263 nm proved the 3*R*,6*R*,3′*R*,6′*R*-configuration **6**. Lactucaxanthin (**6**) is enantiomeric with a tunaxanthin isomer **5** from *Oxyjulis californicus* for which $\Delta\varepsilon$ (EPA) = 9.2 at 268 nm has been reported [1]. The high $\Delta\varepsilon$ value of lactucaxanthin (**6**) compared with those of **3**–**5**, **7** and **8** favours enantiomeric purity.

Lutein (**11**) is a common constituent of several algae and higher plants and has a well-established configuration [7, 17]. Lutein (**11**) from *L. sativa* was identified by co-chromatography, MS and CD data. The carotenoid containing two of the characteristic lutein end groups (D) has previously not been detected. In *L. sativa* lactucaxanthin (**6**) comprised 11% of the total carotenoid and lutein (**11**) was 21%.

The carotenoid pattern of 22 species of Compositae was studied by TLC [11, 12] and electronic spectroscopy. All species contained the typical carotenoid composition of green leaves with β,β-carotene, β,ε-carotene, lutein, violaxanthin (*plus* zeaxanthin generated from violaxanthin under light, see review [18]) and neoxanthin as major components. An additional carotenoid with electronic spectrum and R_f value as for lactucaxanthin was present in all species studied within the closely related [19] genera *Lactuca*, *Prenanthes*, *Cicerbita* and *Mycelis* of the tribe Cichorieae, but was not encountered in the other genera of Compositae examined (Table 1).

* Search for unusual carotenoids in *L. sativa* was initiated by a comment of A. Hager who questioned the concept of different violaxanthin pools in lettuce chloroplasts (Siefermann, D. and Yamamoto, H. Y. (1976) *Plant Physiol.* **57**, 939) and suggested the existence of a pigment not separated from violaxanthin by TLC [12]. No evidence for such a carotenoid was obtained here.

Table 1. Occurrence of carotenoids with an electronic spectrum and R_f value identical with those of lactucaxanthin in Compositae species

Tribe Heliantheae	
Helianthus tuberosus L.	–
Tribe Senecioneae	
Senecio fuchsii C.C. Gmelin	–
Tribe Cichorieae*	
Scolymus group	
Scolymus hispanicus L.	–
Tolpis group	
Hieracium acuminatum Jordan	–
H. piloselloides Vill.	–
Hypochoeris group	
Hypochoeris maculata L.	–
Leontodon autumnalis L.	–
Tragopogon orientalis L.	–
Cichorium group	
A. *Cichorium* subgroup	
C. intybus L.	–
B. *Crepis* subgroup	
Prenanthes purpurea L.	+
Lactuca sativa capitata (L.) DC.	
var. Manoa	+
var. Corelli	+
Lactuca sativa crispa (L.) DC.	+
L. serriola L.	+
L. perennis L.	+
Cicerbita alpina (L.) Wallr.	+
Mycelis muralis (L.) Dumort.	+
Lapsana communis L.	–
Crepis aurea (L.) Cass.	–
Taraxacum officinale Weber	–
Chondrilla juncea L.	–
Launaea arborescens (Batt.) Murb.	–
Sonchus group	
Sonchus asper (L.) Hill	–
S. arvensis L.	–

* Arrangement of genera according to Jeffrey [19].

EXPERIMENTAL

Biological material. L. sativa capitata var. Manoa was grown in a greenhouse for 4 weeks. *L. sativa capitata* var. Corelli (2 months old leaves) was purchased from a local market. Mature leaves of the other species were obtained from the Botanical Garden of Tübingen or were collected at nearby locations in September 1979.

For analytical studies, 0.2–0.4 g of leaf material was homogenized in a mortar together with 0.5 g $CaCO_3$ and 5 ml Me_2CO; the Me_2CO extract was concd and aliquots containing *ca* 15 μg of carotenoids were subjected to TLC. For preparative extraction of pigments, chloroplasts of *L. sativa capitata* var. Corelli were used. Chloroplasts were isolated by homogenization of green leaves (250 g) in 300 ml of medium (300 mM sorbitol, 10 mM NaCl, 50 mM Na-phosphate buffer pH 7.5), centrifugation at 3000 *g*, osmotic shock in distilled water and centrifugation at 6000 *g*.

Methods. General precautions for work with carotenoids were taken. TLC was carried out either on lipid-impregnated kieselguhr plates [12] with MeOH–Me_2CO–H_2O (20:4:3), on freshly-activated alkaline plates [11] with Me_2CO–petrol (bp 110–140°)–$CHCl_3$ (70:45:10) or on mixed MgO plates [14] with hexane–Me_2CO (70:30). HPLC was carried out as described elsewhere [15].

Lactucaxanthin (**6**) ex Lactuca sativa. Chloroplasts equivalent to 50 mg chlorophyll were quantitatively extracted with Me_2CO; the extract was concd and preparatively separated on lipid-impregnated plates [12]. (**6** + **11**) was eluted in EtOH, concd and frozen to remove the co-eluted fat coating. In the reversed phase system (**6** + **11**) was more strongly adsorbed than **12**. Upon rechromatography on alkaline plates [11] **6** and **11** separated, **5** being less strongly adsorbed. A mixture of **6** and **11** was preparatively separated on the MgO plates [15], again **6** was less strongly adsorbed. Available for further studies was 0.3 mg **6**. Compound **6** had $\lambda_{max}^{Me_2CO}$ nm: 426, 440 and 471, % III/II [16] = 93; λ_{max}^{EtOH}: 424, 438.5 and 468, % III/II = 90; MS *m/e*: 568 (M^+), M − 18, M − 18 − 18, M − 92 and M − 106 (only diagnostically useful MS peaks are cited); ^{1}H NMR ($CDCl_3$, 100 MHz FT): δ 0.85 (6 H, *s*), 1.00 (6 H, *s*), 1.62 (6 H, *s*), 1.91 (6 H, *s*), 1.96 (6 H, *s*), *ca* 5.4 (2 H, *m*) and 6.04–6.65 (*ca* 14 H); CD (EPA) nm (Δε): 215 (+4), 230 (+7), 264 (+17.5), 290 (+1.5), 325 (+2.5), 390 (0). Δε values are based on $E_{1cm}^{1\%} = 2500$ at $\lambda_{max}^{Me_2CO}$ for **6**. Treatment of **6** with *ca* 0.3 N HCl in EtOH caused a 2 nm hypochromic shift of λ_{max}. In a parallel experiment with violaxanthin (**12**), λ_{max} shifted from 416, 440 and 470 nm to 380, 401 and 426 nm; % III/II = 93 as expected for **13**.

Lactucaxanthin monomethyl ether (**14**). To **6** (70 μg) in $CHCl_3$ (0.1 ml) and MeOH (3 ml) was added $CHCl_3$ saturated with dry HCl (0.3 N) to a final acid concn of 0.03 N. After 3 hr at room temp. the mixture contained unreacted **6** (R_f = 0.45, Si gel plates, hexane–Me_2CO, 7:3; 60% of total), the monomethyl ether **14** (R_f = 0.71; 30% of total) and the dimethyl ether **15** (R_f = 0.93; 10% of total). The reaction mixture was ketp at +6° overnight; pigment recovery after transfer to Et_2O was 69% consisting of **6** (10% of total), **14** (60%) and **15** (30%). Compound **14** had an electronic spectrum as for **6**; MS *m/e* 582 (M^+), M − 18, M − 18 − 32.

Lactucaxanthin dimethyl ether (**15**) had an electronic spectrum as for **6**; MS *m/e*: 596 (M), M − 32, M − 32 − 32, M − 32 − 32 − 106.

Lutein (**11**) ex Lactuca sativa could not be separated from authentic **11**; $\lambda_{max}^{Me_2CO}$ nm: (430), 445 and 473 nm, % III/II = 69; MS *m/e* 568 (M^+), M − 18, M − 18 − 18, CD (EPA) nm (Δε): 263 (+7.5, 300 (0), 332 (÷3), 355 (0), 385 (+1), 390 (0).

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