140. Reinvestigation of Original Taraxanthin Samples[1])

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Überprüfung einiger Originalpräparate von Taraxanthin. – *Zusammenfassung.* Das von *R. Kuhn & E. Lederer* 1931 entdeckte und seither in zahlreichen höheren Pflanzen nachgewiesene Taraxanthin wird anhand von Originalpräparaten aus *Impatiens nolitangere* L., *Ranunculus acer* L. sowie durch erneute Isolierung aus *Taraxacum officinale* L. mit modernen Methoden untersucht. Taraxanthin erweist sich als Gemisch von Xanthophyll-epoxid (= Lutein-epoxid; **2**) oder dessen Umlagerungsprodukten und O_4-Carotinoiden bekannter Struktur. Das aus *Taraxacum* erhaltene Präparat setzt sich aus **2** (73%), Flavoxanthin (**4**, 13%) und Chrysanthemaxanthin (**4** (C(8)-Epimer), 14%) zusammen. Nicht identifizierte Carotinoide in den untersuchten Präparaten betragen weniger als 3,5%. Ein in kleiner Menge in *R. acer* nachgewiesenes Carotinoid ist wahrscheinlich 5,6-Dihydroxy-5,6-dihydrolutein (**6**).

Der Name Taraxanthin als Bezeichnung für ein einheitliches Carotinoid soll aufgegeben werden.

Taraxanthin was first characterized in 1931 by *Kuhn & Lederer* [1] from *Taraxacum officinale*: m.p. 185°; $C_{40}H_{56}O_4$ by combustion; uptake of 10.65 mol H_2 on catalytic hydrogenation; 3.25 active H; no blue colour with 25% HCl-solution. Subsequent isolations of crystalline taraxanthin from other plants [2] were reported by the schools of *Kuhn* [3] [4], *Karrer* [5–7], *Zechmeister* [8] and *Heilbron* [9]. Additional claimed sources have been reviewed [10].

In 1957 *Eugster & Karrer* [10] reisolated taraxanthin (m.p. 184°, $C_{40}H_{56}O_4$) from *Impatiens nolitangere*, one of the better sources reported by *Kuhn & Lederer* [3], and revealed its epoxide nature by detailed studies of the colour reaction with HCl and conversion to the furanoid tarachrome. Taraxanthin was considered to be a hydroxy derivative of lutein epoxide.

From a reexamination of several classical sources of taraxanthin in 1968 *Egger* [11] has claimed the identity of taraxanthin with lutein epoxide[2]) (**2**), $C_{40}H_{56}O_3$, on the basis of electronic spectra and chromatographic properties alone. This conclusion was supported by similar findings, including IR. properties, of *Tóth & Szabolcs* [12]. Attempts by *Cholnoky et al.* [13] to reisolate taraxanthin from *Taraxacum officinale* and *Impatiens nolitangere* only resulted in lutein epoxide (shown by MS.).

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[1]) No. 10 in the Trondheim series Carotenoids of higher plants. No. 9: Phytochemistry *14*, 797 (1975).

[2]) = 5,6-Epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol.

Later *Nitsche & Pleugel* [14] claimed identity of the allenic neoxanthin ($C_{40}H_{56}O_4$) *ex Taraxacum officinale* and *Impatiens nolitangere* with taraxanthin.

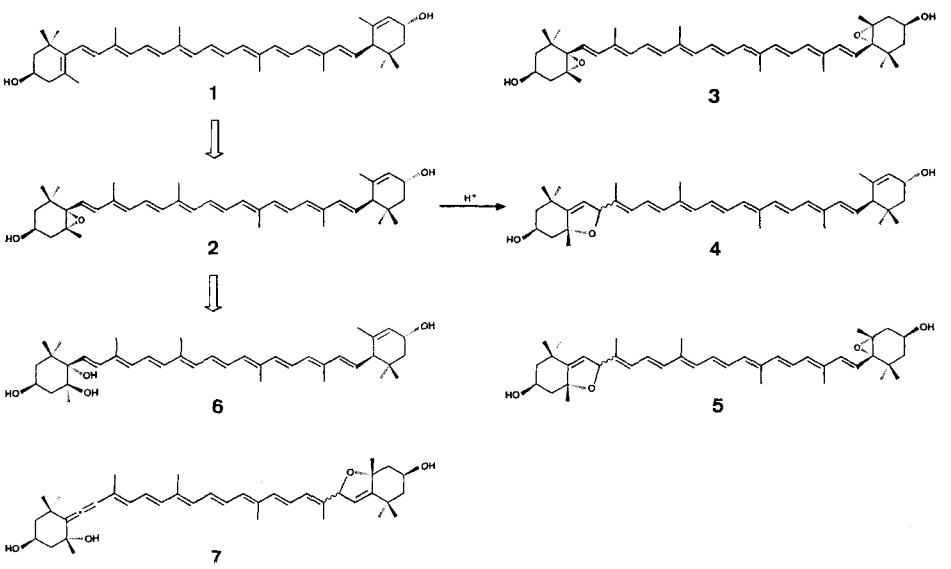
In view of the many unsuccessful attempts to reisolate taraxanthin, it was desirable to reexamine previously isolated samples of taraxanthin by modern methods. A taraxanthin sample from the sample collection of *Richard Kuhn* was already reported to contain an unknown quantity of a $C_{40}H_{56}O_4$ constituent by mass spectroscopy [13].

In the present study a quantitative study of crystalline taraxanthin *ex Impatiens nolitangere* and *Ranunculus acer* from *Karrer's* collection is reported. A freshly isolated sample *ex Taraxacum officinale* was also checked.

Preliminary chromatographic comparison of crystalline taraxanthin samples *ex Impatiens nolitangere* and North American *Impatiens sp.* revealed mixtures of the same qualitative composition. The taraxanthin sample *ex Ranunculus acer* differed in qualitative respect.

1. Taraxanthin from *Impatiens nolitangere*. – The following carotenoids (in order of increasing polarity) were identified by means of VIS. spectra and MS., co-chromatography and specific chemical reactions such as acid-catalysed epoxide-dihydrofuran rearrangement, acetylation, silylation, allyl ether formation in combination with the MS. of the products formed: unidentified material (0.5%), lutein[3]) (**1**; 1.7%), lutein epoxide[2]) (**2**; 76,8%), violaxanthin[4]) (**3**; 13.1%), flavoxanthin/chrysanthemaxanthin[5]) (**4**, two C(8)-isomers; 2,7%), and luteoxanthin[6]) (two C(8)-

Scheme



[3]) = β,ε-Carotene-3,3′-diol.

[4]) = 5,6:5′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β,β′-carotene-3,3′-diol.

[5]) = 5,8-Epoxy-5,8-dihydro-β,ε-carotene-3,3′-diol.

[6]) = 5,8:5′,6′-Diepoxy-5,8,5′,6′-tetrahydro-β,β′-carotene-3,3′-diol.

isomers) (**5**; 5.2%); *i.e.* 79.5% O_3-compounds, 18.3% O_4-compounds and 2.2% others. For the individual identifications, see Exper. part.

2. Taraxanthin from *Ranunculus acer*. – By the criteria described above the following composition was demonstrated: lutein[3]) (**1**; 57.8%), flavoxanthin/chrysanthemaxanthin[5]) (**4**; 30.9%), 5,6-dihydroxy-5,6-dihydro-lutein (**6**; 5.1%), trihydroxy carotenoid (2.1%), unknown O_4-carotenoid (1.4%) and neochrome[7]) (**7**; 2.7%). The complete absence of lutein epoxide (**2**) in this sample may indicate that it has been quantitatively rearranged to flavoxanthin/chrysanthemaxanthin (**4**) during isolation or storing.

Compound **6** represents a new carotenoid which is more polar than lutein epoxide (**2**). It has a molecular weight of 602 by MS., compatible with $C_{40}H_{58}O_4$, and its electronic spectrum is similar to the one of **2**. It suffered no epoxy rearrangement on acidic treatment, but gave furylium and homopyrylium ions on electron impact and formed a diacetate upon acetylation which could not be silylated. This evidence is consistent with structure **6**. Failure of **6**-diacetate to form a trimethylsilyl ether is compatible with a (3,5-*cis*, 5,6-*trans*)-relationship of the hydroxy groups in the cyclohexane ring as judged from recent studies on heteroxanthin [15]. **6** may be considered a metabolite of lutein epoxide (**2**). Similar metabolites of antheraxanthin and violaxanthin were recently reported by *Gross et al.* [16].

The trihydroxy carotenoid (2.1%) has the lutein (**1**) chromophore and contains three secondary or primary hydroxy groups as shown by formation of a triacetate. The evidence is insufficient for further assignments.

The unknown O_4-carotenoid (1.4%) also has the lutein (**1**) chromophore, and a molecular formula $C_{40}H_{56}O_4$ by MS. It formed a diacetate on acetylation and no epoxy rearrangement product on acidic treatment.

3. Taraxanthin from *Taraxacum officinale*. – The virtually homogenous crystals, isolated by the procedure described by *Kuhn* [1] and analysed by visible spectra and co-chromatography with authentic samples, consisted of lutein epoxide (**2**; 73%), flavoxanthin (**4**, C(8)-isomer; 13%), and chrysanthemaxanthin (**4**, C(8)-isomer; 14%).

4. Conclusions. – The present reexamination of authentic taraxanthin samples confirms that taraxanthin is a mixture containing lutein epoxide (**2**) as the major component. This is consistent with most properties reported in the literature (m.p., electronic spectrum, hydrogenation, *Zerewitinoff* determination and rearrangement to a dihydrofuran-derivative). The presumed content of four oxygen atoms in taraxanthin may be explained by some admixture with O_4-carotenoids and analytical errors.

This conclusion is confirmed by the optical activity of the carotenoids under consideration. Reported $[\alpha]_{Cd}^{20}$ values are: lutein (**1**) +145° [17], lutein epoxide (**2**) +225° [18] and taraxanthin +200° [1]. The strong positive rotation of taraxanthin confirms the presence of the ε-ring, since no other end group in the carotenoid series makes such a strong contribution at the Cd line.

The identity, also in stereochemical respect, of lutein epoxide (**2**) *ex Impatiens nolitangere* and lutein epoxide *ex Taraxacum officinale* studied by *Cadosch & Eugster* [19] follows from our CD. measurements (see Exper. part.)

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[7]) = 5′,8′-Epoxy-6,7-didehydro-5,6,5′,8′-tetrahydro-β,β-carotene-3,5,3′-triol.

The isolation of taraxanthin from *Taraxacum officinale* was carried out by dipl. chem. *H. Cadosch*, Universität Zürich. *R. B.* thanks the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* for a postdoctoral fellowship.

Experimental Part

General. For chromatographic analysis and separation of the crystalline samples 1 mm thin layer plates precoated with a mixture of silica gel/$Ca(OH)_2$/MgO/$CaSO_4$ 10:4:3:1 or silica gel alone was used. Elution was effected with acetone/hexane 3:2 and 3:7, respectively. In difficult cases the acetate was formed for final purification. Rf values refer to circular paper *Whatman* SG 81 (= SG 81) and acetone/hexane (AHE) 26:74 as eluent if not specified. Visible spectra were measured in ether (λ_{max} in nm). Mass numbers in the MS. are given in *m/e*. Other materials and methods were as usually employed in the Norwegian laboratory and are summarized elsewhere [20].

1. *Taraxanthin* ex Impatiens nolitangere [10]. For crystalline taraxanthin (19.4 mg) the following constituents were established:

Lutein (**1**). 0.31 mg, Rf = 0.60, no separation on chromatography with an authentic sample. – VIS.: 472.5, *447*, 422. – MS.: 568 (*M*), 550 (*M* – 18), 476 (*M* – 92), 458 (*M* – 18–92).

Formation of the 3′-methyl ether (Rf = 0.76) on treatment with 0.388 N HCl in methanol, no changes in VIS. spectrum.

Lutein epoxide (**2**). 14.90 mg, Rf = 0.55. – VIS.: 470, *440*, 416. – MS.: 584 (*M*), 566 (*M* – 18), 550 (*M* – 18–16), 504 (*M* – 80), 492 (*M* – 92), 486 (*M* – 18–80), 474 (*M* – 18–92), 211 (homopyrylium), 181 (furylium). – CD. ($\Delta\varepsilon$, dioxane): 231 (+7.6), 262 (−2.5), 271 (−5.6), 331 (+3.8) nm.

On treatment with 0.388 N HCl in methanol formation of the 3′-methyl ether accompanied by the epoxy rearrangement to a dihydrofuran derivative: Rf = 0.73. – VIS.: 449, *423*, 400.

Violaxanthin (**3**). 2.54 mg, Rf = 0.44, no difference on co-chromatography with an authentic sample. – VIS.: 470, *440*, 417. – MS.: 600 (*M*), 584 (*M* – 16), 582 (*M* – 18), 566 (*M* – 16–18), 520 (*M* – 80), 502 (*M* – 92), 504 (*M* – 16–80), 502 (*M* – 18–80), 440 (*M* – 160), 221 (homopyrylium), 181 (furylium).

On treatment with 0.388 N HCl in methanol no ether formation but rearrangement to a bis(dihydrofuran) derivative (= auroxanthin). – VIS.: 426, *400.5*, 380.

Flavoxanthin/chrysanthemaxanthin (**4**) (*as isomeric mixture*). 0.52 mg, Rf = 0.60. – VIS.: *448.5*, 421.5, 398. – MS.: 584 (*M*), 566 (*M* – 18), 504 (*M* – 80), 492 (*M* – 92), 486 (*M* – 18–80), 474 (*M* – 18–92), 221 (homopyrylium), 181 (furylium).

Luteoxanthin (*two C(8) isomers*) (**5**). 1.01 mg; Rf = 0.47. – VIS.: 449, *421.5*, 399. – MS.: 600 (*M*), 584 (*M* – 16), 568 (*M* – 16–16), 566 (*M* – 16–18), 520 (*M* – 80), 504 (*M* – 16–80), 221 (homopyrylium), 181 (furylium).

Rearrangement to auroxanthin on treatment with HCl in ether: Rf = 0.49. – VIS.: 425, *401*, 381.

2. *Taraxanthin from* Ranunculus acer [6]. 4.3 mg crystalline taraxanthin gave on separation:

Lutein (**1**). 2.34 mg, Rf = 0.60. – VIS.: 473.5, *445*, 421.5. – MS.: 568 (*M*), 550 (*M* – 18), 476 (*M* – 92), 458 (*M* – 18–92).

Flavoxanthin/chrysanthemaxanthin (**4**). 1.25 mg, Rf = 0.60. – VIS.: 447.5, *421.5*, 399. – MS.: 584 (*M*), 566 (*M* – 18), 504 (*M* – 80), 492 (*M* – 92), 486 (*M* – 18–80), 474 (*M* – 18–92), 221 (homopyrylium), 181 (furylium).

5,6-Dihydroxy-5,6-dihydro-lutein (**6**). 0.207 mg, Rf = 0.16. – VIS.: 469, *439.5*, 416. – MS.: 602 (*M*), 584 (*M* – 18), 221 (homopyrylium), 181 (furylium).

Diacetate: Rf (AHE 1:4) = 0.69, cannot be silylated, no rearrangement with HCl in ether.

Trihydroxy carotenoid as triacetate. 0.087 mg, Rf (AHE 1:4) = 0.76 (Rf (free alcohol) = 0.36). – VIS.: 472, *445*, 423. – MS.: 710 (*M*), 650 (*M* – HAc), no homopyrylium or furylium ions.

On silylation or treatment with HCl in ether no reaction.

Unknown O_4-carotenoid (acetylated). 0.056 mg, Rf (AHE 1:4) = 0.71 (Rf (free alcohol) = 0.25). – VIS.: 471, *442.5*, 421. – MS.: 684 (*M*), 668 (*M* – 16), 624 (*M* – HAc), 263 (homopyrylium), 223 (furylium).

On silylation or treatment with HCl in ether no reaction.

Neochrome (**7**). 0.111 mg, Rf (AHE 1:4) monoacetate, = 0.44, Rf (diacetate, AHE 1:4) = 0.57, no difference in co-chromatography with authentic neochrome diacetate. – VIS.: *449*, 422, 399. – MS. (monoacetate): 642 (*M*), 626 (*M* – 16), 624 (*M* – 18), 608 (*M* – 16–18), 562 (*M* – 80), 550 (*M* – 92), 544 (*M* – 18–80), 221 (homopyrylium), 181 (furylium).

Diacetate monosilylether: Rf (SG 81, AHE 1:9) = 0.64 and 0.71 (2 isomers). – MS.: 756 (*M*), 676 (*M* – 80), 666 (*M* – $(CH_3)_3SiOH$), 664 (*M* – 92), 606 (*M* – 90 – HAc), 586 (*M* – 90–80), 263 (homopyrylium), 223 (furylium).

3. *Taraxanthin from* Taraxacum officinale. Fresh isolation as described in [1] gave crystalline taraxanthin with m.p. 184.5°. – VIS.: 471, *445* (ε = 146000), 420.

After separation on thin layer plates the following compounds were identified by co-chromatography and visible spectra:

Lutein epoxide (**2**). – VIS.: 470, *440*, 417.

Flavoxanthin (**4**). – VIS.: *448*, *421*, 398.5.

Chrysanthemaxanthin (**4**):. – VIS.: *448*, *421*, 398.5.

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