

Carotenoid structures, old and new problems

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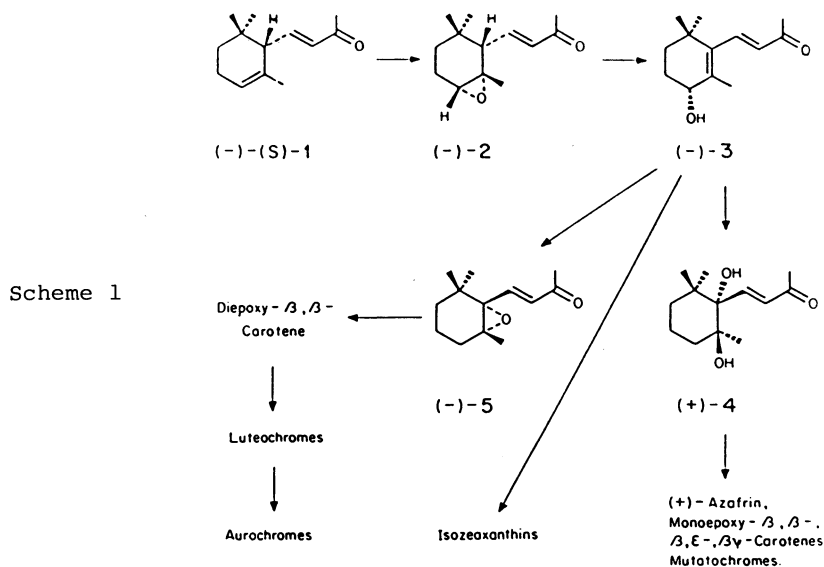
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Abstract - Optically active epoxy-ionones and epoxy-carotenoids and their solvolytic conversions to glycols. Syntheses of 3,5,6-trihydroxy-5,6-dihydro- β -endgroups and derived carotenoids. Isolation and structures of 12 novel carotenoids from petals of *Rosa foetida*: stereoisomeric luteoxanthins, auroxanthins, latoxanthins and latochromes. Revision of the published structures of heteroxanthin, mactraxanthin and other carotenoids having 3,5,6-trihydroxy- β -endgroups.

During the past few years we have worked on carotenoid epoxides and achieved several results which I hope will also be of interest to the members of our carotenoid club.

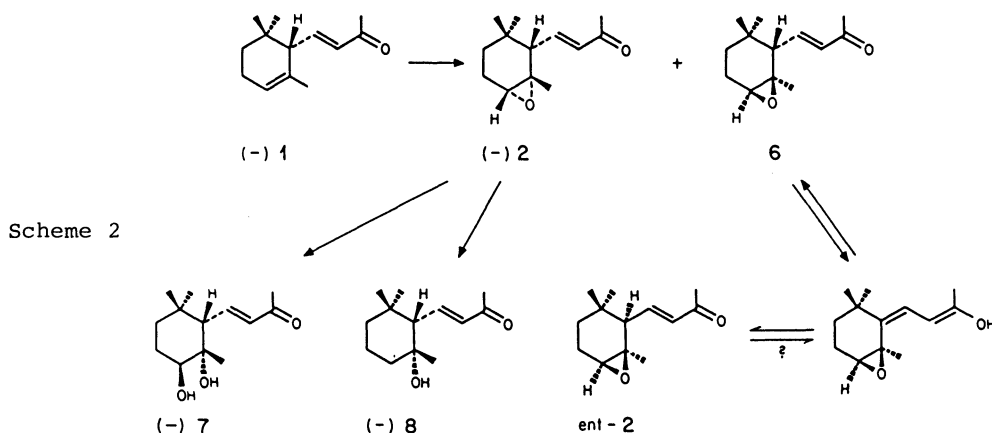
Carotenoid epoxides have their particular and characteristic value which although not yet fully recognised in all dimensions has, however, been amply recorded by evidence of their ubiquitous distribution and function in the violaxanthin cycle.

For us, in addition, further and very specific aspects of epoxide-reactions gradually became apparent; See Scheme 1.



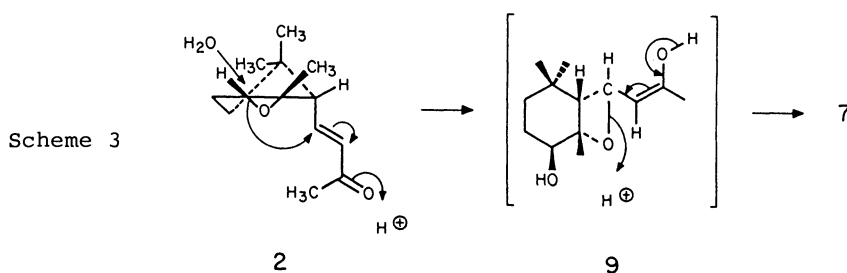
Since an earlier investigation by Ohloff and Mignat [1] it has been assumed that the epoxidation of α -ionone proceeds stereoselectively - on optically active α -ionone (1) this leads to *cis*-epoxide 2, as we have shown. Subsequently we have used this compound for numerous structural correlations e.g. for the synthesis of the optically active ionone derivatives 3, 4, and 5 which, in their turn, served as starting materials for the synthesis of several optically active carotenoids such as isozeaxanthin [2], azafrin [3], monoepoxy- β, β -carotene [4], monoepoxy- β, ϵ - and β, ψ -carotenes [5], mutatochromes [6], diepoxi- β, β -carotene [7], luteochromes [8] and aurochromes [9].

The assigned absolute configurations depend, therefore, on the correctness of structure 2. It thus became a priority for us to verify by direct methods the configuration assigned by Ohloff on the basis of indirect evidence, see Scheme 2



The first surprise resulted when GC-analyses showed that in reality no selective epoxidation occurs at all; two diastereomeric epoxides being formed in the ratio of 4:1, although, of course, the question remained open as to whether the *cis*- or *trans*-epoxide dominated. The lower-yield epoxide was very labile and rearranged easily to the main compound. Our surprise was complete, when X-ray analysis of the hydrolysis product, a) led to structure 7, since with 2 as starting material "a priori", inversion at C(5) would be expected.

A further X-ray analysis on an alcohol formed by reduction of the epoxide with LiAlH_4 indicated structure 8. From this it now follows with certainty that the main product is indeed the *cis*-epoxide 2. An interpretation for the unexpected epoxide hydrolysis is given b) in Scheme 3.



Protonation occurs at the side-chain carbonyl initiating a neighbouring group participation of the epoxy-function. The diol 7 is then formed via the strained vinyl-oxetane 9, inversion of the epoxide resulting at the secondary and not at the tertiary centre.

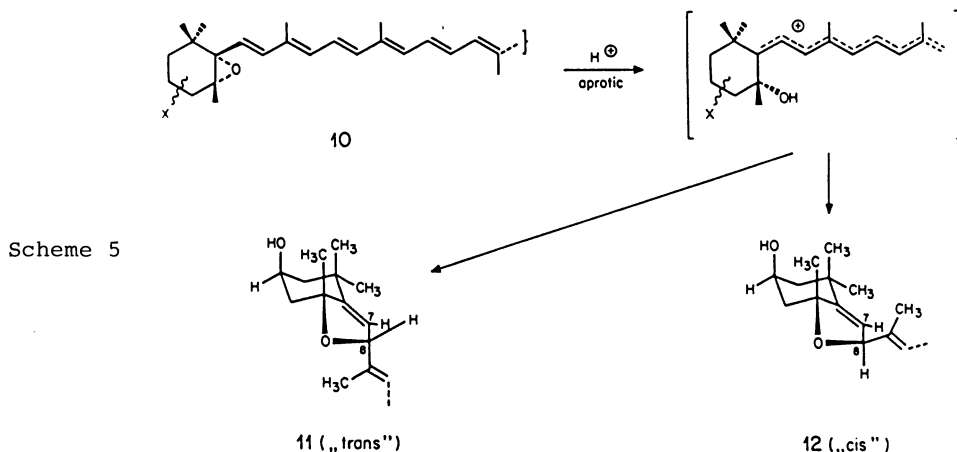
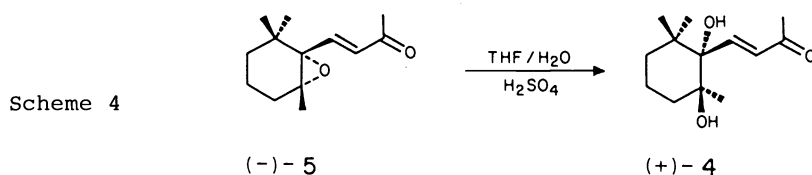
This assures that the basis for our structure correlations is correct.

If one hydrolyses optically active β -ionone epoxide (5), see Scheme 4, the weaker C(5)-O bond is broken, in accordance with the textbooks, and enantiomeric pure *trans*-glycol 4 is formed, i.e. inversion occurs here at C(5).

Although I have just described to you over half a dozen new optically active ionone endgroups I do not wish to discuss their use, but would like to turn to reactions on carotenoid epoxides since they offer us more surprises.

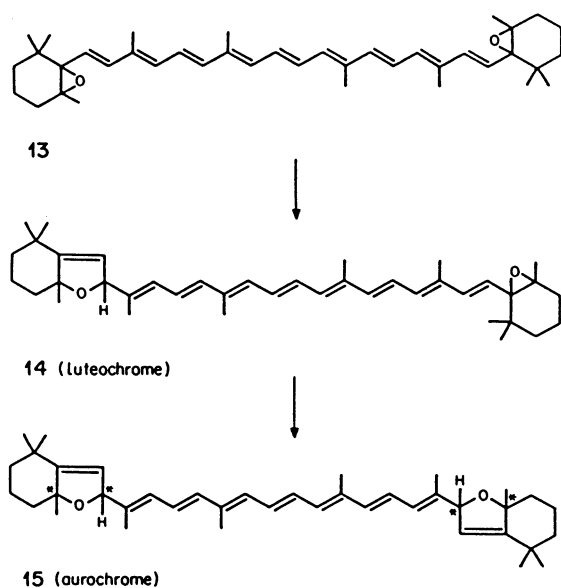
a) Carried out on the original product from [10].

b) My thanks to Prof. Jack Baldwin, Oxford University, for a discussion on the possible mechanisms.



As you know, 5,6-epoxy-carotenoids e.g. 10 under aprotic conditions rearrange into furanoid-5,8-epoxides, the configuration at C(5) remaining unchanged [3], [11 - 14]. Till today there are no exceptions to this rule. In future, however, possible neighbouring group participation by a group X in 10 should not be neglected.

The new centre of chirality at C(8) predicts the formation of two diastereomers, namely 11 and 12. In most cases, clear separation has only been made possible by modern HPLC-methods. I would like to illustrate this by an apparently simple example:



Scheme 6

You all know the constitution of β,β -carotene diepoxide (13) and its rearrangement products luteochrome and aurochrome. Stereochemical consideration indicates that "aurochrome" can exist as 10 stereoisomers, namely as 4 pairs of enantiomers and two meso-forms. What is meant then, when in recent publications on the analysis of carotenoids from some organism or other we see only "aurochrome" written? Actually, a differentiation between the 10 stereoisomers is a delicate task and can only be obtained by a combination of HPLC, NMR and CD [9].

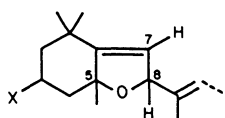
Today the following $^1\text{H-NMR}$ criteria can be listed for differentiation of the cis- and trans-substitution on the dihydrofuran ring; see Scheme 7.

These rules are based on measurements on numerous diastereomers of furanoid carotenoids [6], [7], [12], [15], [16].

In all HPLC-analyses of natural 5,8-

epoxides always both C(8)-epimers were found. When, therefore, newer publications report only evidence of a single diastereomer e.g. flavoxanthin without chrysanthemaxanthin, this is in my view hardly correct.

Scheme 7



X = H, OH, OAc

cis - CH₃ (5) / C(9):
 $\Delta \delta$ H-C(7)/H-C(8) = 0,15-0,22 ppm
 H-C(7) d, ³J > 1,4 Hz.

trans - CH₃ (5) / C(9):
 $\Delta \delta$ H-C(7)/H-C(8) < 0,09 ppm
 H-C(7) s broad

I would like to illustrate this by means of an analysis of the carotenoids from *Rosa foetida*; see Figure 1.

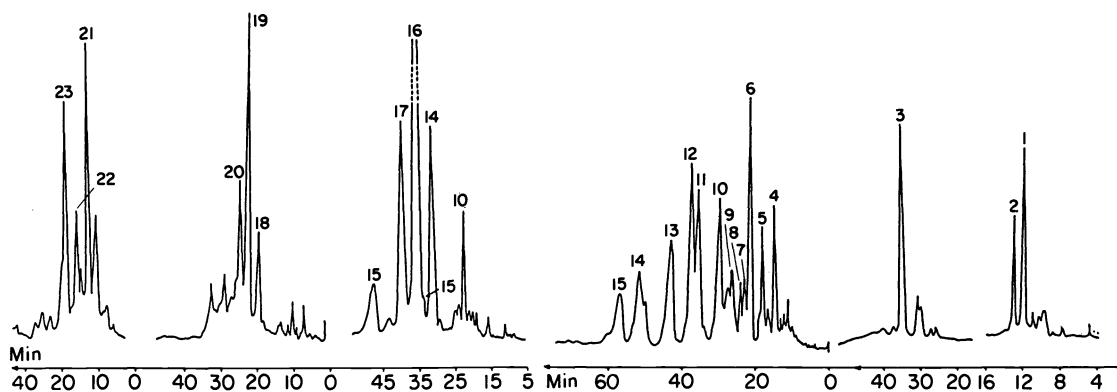
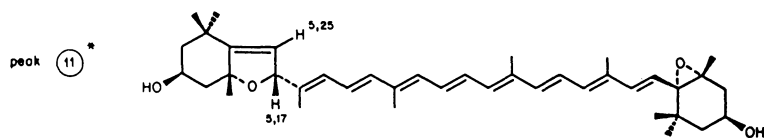
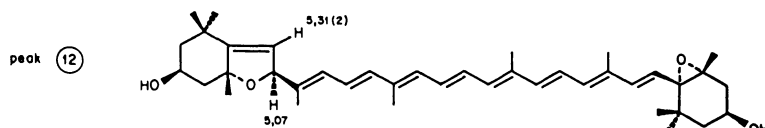


Figure 1

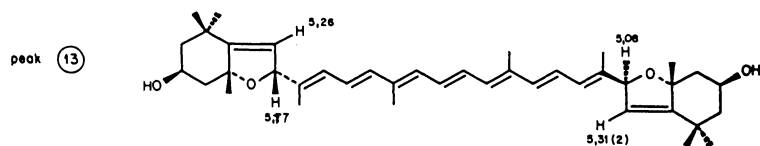
peak ⑨ unknown (Z) - auroxanthin



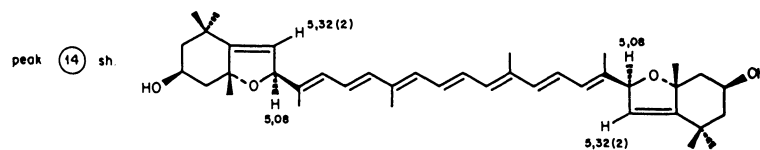
16 (8R)-Luteoxanthin



17 (8S)-Luteoxanthin

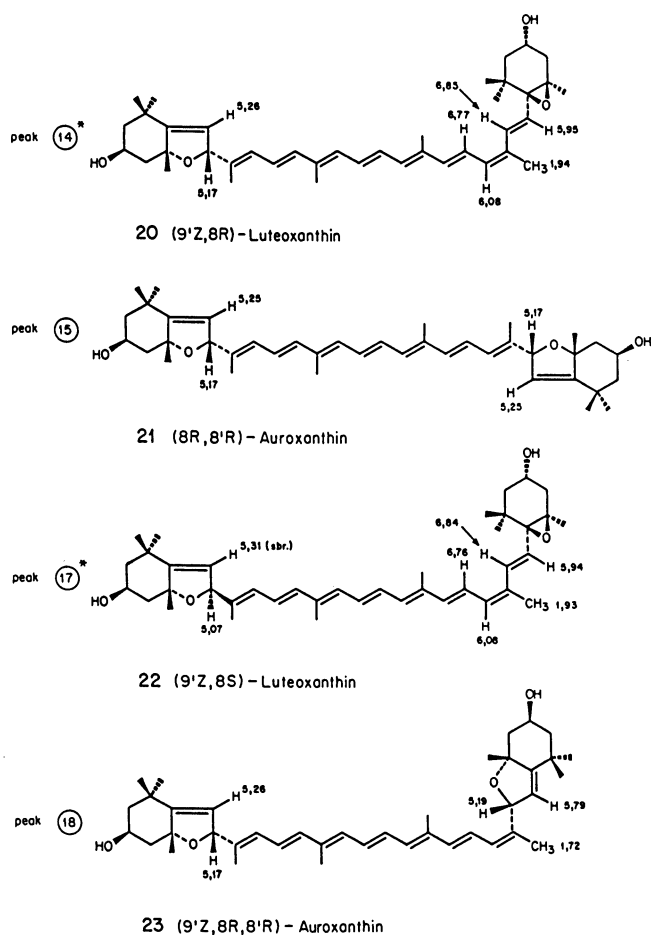


18 (8R,8'S)-Auroxanthin



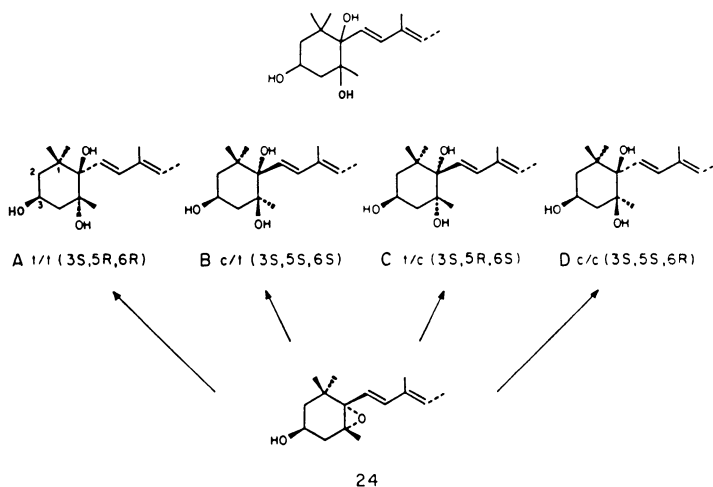
19 (8S,8'S)-Auroxanthin

Scheme 8



Scheme 9

pounds are present because peak 21 can be separated into two components by another system. On the strength of NMR- and mass-spectra we came to the conclusion that carotenoids with triol endgroups are present which do not correspond though with any of those already known. However, their properties point in the direction of those highly polar carotenoids from orange-juice first described under diverse names by Curl [17] and later by Mrs. Gross [18].



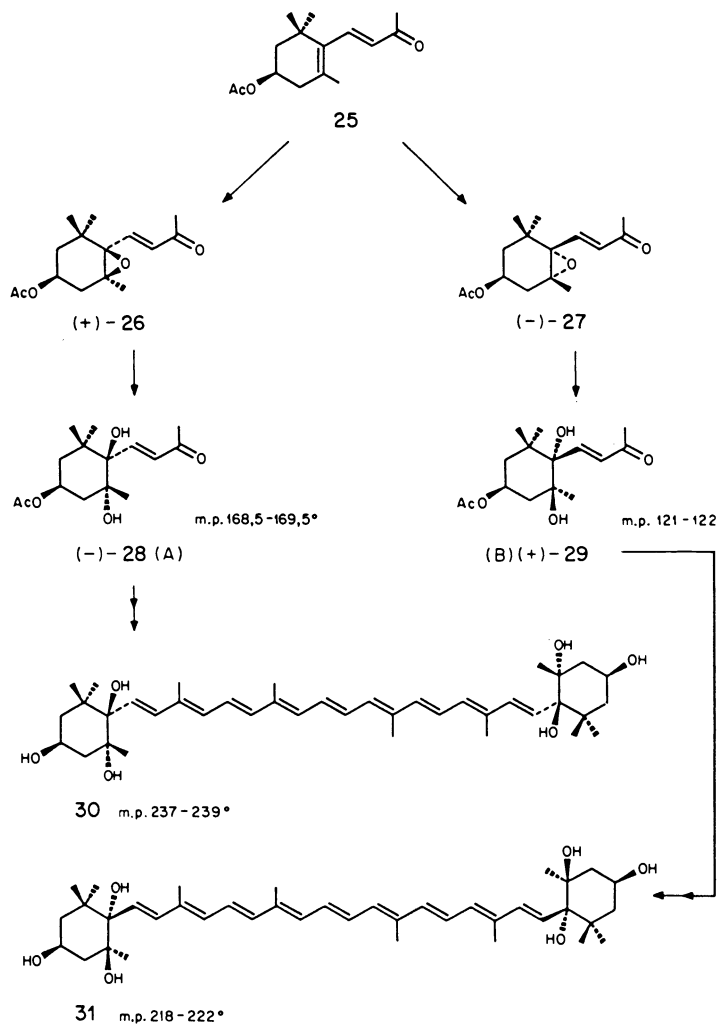
Scheme 10

Here a more recent HPLC-separation of 6 crude fractions from a ZnCO_3 -column chromatography is depicted. Epiphasic carotenes have been omitted. In the first epoxide fraction we found 8 carotenoids, whose names though long known in the literature have structurally never been precisely described; see Schemes 8 and 9. It concerns the four luteoxanthins 16, 17, 20, 22 and the four auroxanthins 18, 19, 21, 23. In addition further (Z)-isomers exist which we don't yet know. Of these 8 new carotenoids 16, 20 and 22 (in addition to violaxanthins) contribute most to the brilliant yellow hue.

These examples illustrate the level of carotenoid analysis possible today and the degree of structural refinement that one can attain. I do not wish to dwell upon the neoxanthin-neochrome fraction here.

Of considerably greater interest is the fraction which is more polar than the neoxanthin fraction. On classical column chromatography it remains at the top together with the brown decomposition products. At this point I would like to draw your attention to the nature of the peaks 21, 22 and 23. In actual fact, 4 new com-

If one postulates the constitution of a 3,5,6- β -triol endgroup, one can visualize its formation from the violaxanthin endgroup (24). From thence the triol endgroups A - D can be derived; see Scheme 10. But their structural elucidation from spectra alone appeared to us very difficult, and we also expected surprises from the epoxide hydrolyses due to possible neighbouring group participation of the OH at C(3). For this reason we first of all decided to build up these endgroups by an unequivocal synthetic route; see Scheme 11.



Epoxidation of the well-known (R)-3-acetoxy- β -ionone [19] gave 26 and 27 [20]. Hydrolysis led to triols, whose structures 28 and 29 were each established by X-ray analysis. Thus we had the optically active endgroups A and B in our hands. Evidently this time no neighbouring group participation occurred on epoxide hydrolysis. According to known methods the carotenoids 30 and 31 were then built up. They distinguish themselves not only by their melting points but also by great differences in polarity, silylating ability at OH-C(5) etc.

In order to also obtain the endgroups C and D, we mildly hydrolysed lutein epoxide (32) on the one hand and epilutein epoxide (33) on the other; see Scheme 12.

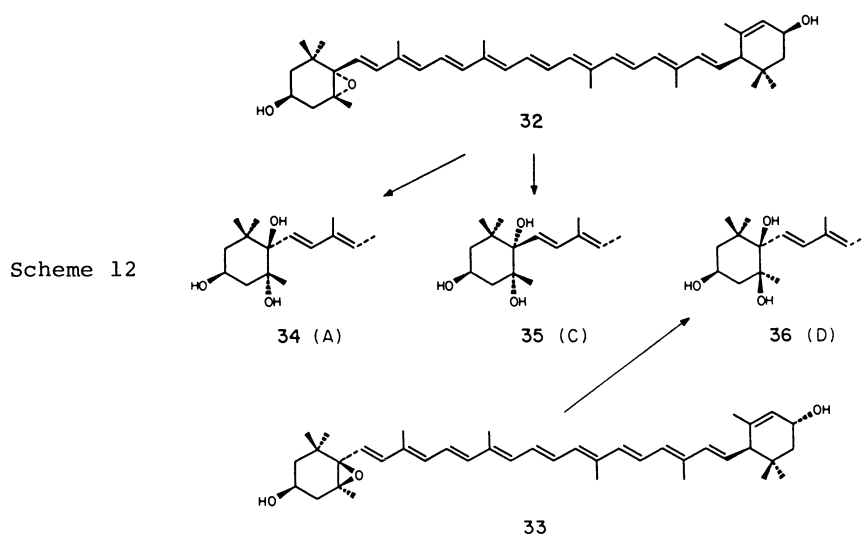
From 32 two polar tetrols were formed, one of which (according to NMR) possesses the endgroup A and thus has structure 34. The other was different from A and B. If one does not assume inversion at both centres one arrives at structure 35 with endgroup C. Analogously from semi-synthetic epilutein epoxide (33) a further endgroup was obtained which was different from that of A, B and C and consequently represents the last possibility, namely D.

It must be emphasized that the triol endgroups once formed are stable and under hydrolytic conditions not interconvertible.

From these results it follows that the hydrolysis of carotenoid 5,6-epoxides proceeds under retention of configuration at C(5)! At C(6) either inversion or retention can occur.

In Scheme 13 NMR-data are reproduced which show that the methyl signals, despite lying closely can be clearly distinguished at 200 MHz, thereby allowing a structural assignment with relative stereochemistry.

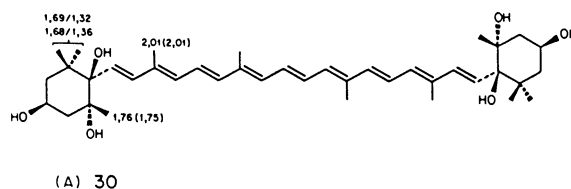
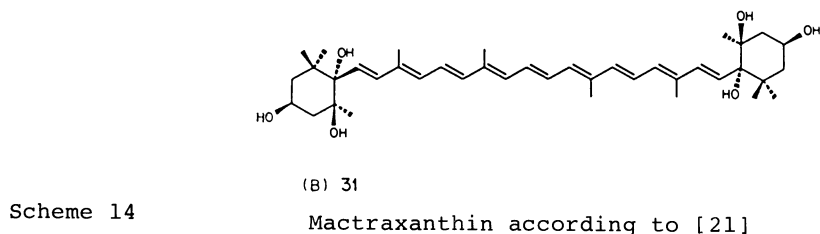
If we compare the data determined on our synthetic product with those of mac-traxanthin, a new carotenoid from *Maत्रaxanthin chinensis* [21] (see Scheme 14), it is evident that its structure must be revised. Maत्रaxanthin has endgroup A with a trans-trans-stereochemistry of the hydroxyls and not cis-trans as published.



Scheme 13

	A	B	C	D
16	0,87	0,90	0,82	1,03
17	1,17	1,13	1,11	1,06
18	1,24	1,31	1,27	1,35
19	1,96	1,98	1,92	1,94

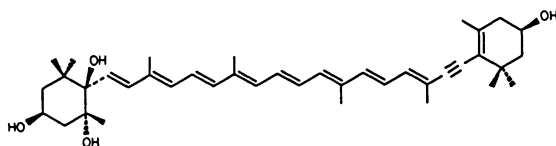
Chemical shifts of methyls in endgroups A,B,C,D
(200 MHz, CDCl₃)



Mactraxanthin according to this paper, see [22]
(200 MHz, d₅-Pyridin [21][22])

Further revisions have also become necessary for other carotenoids with triol endgroups; see Scheme 15.

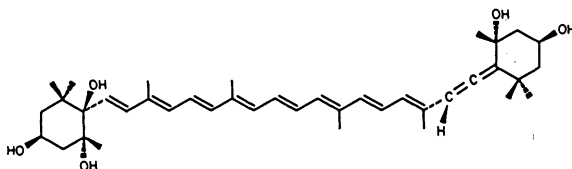
For heteroxanthin a structure with endgroup B has been earlier proposed [23]. Based on the new criteria we alter it to 37. This also applies to a polar pentaol from *Trollius europaeus*. It is a hydrolysis product of neoxanthin [24]. We now assign to it structure 38. The less polar pentaol is correctly formulated with endgroup C.



(A) 37

Scheme 15

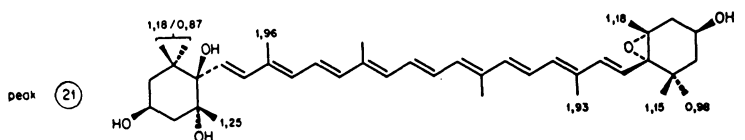
Heteroxanthin according to [22]



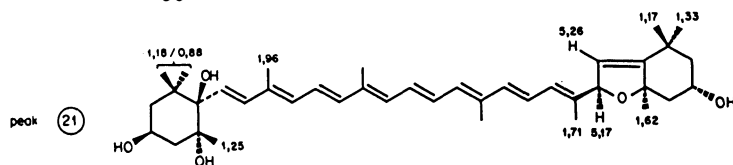
(A) 38

Polar pentaol from Trollius europaeus according to [22]

Now to our new polar carotenoids from yellow roses. On the basis of detailed NMR-comparisons the following structures with relative stereochemistry result; see Scheme 16.

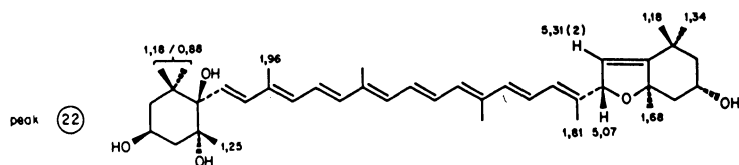


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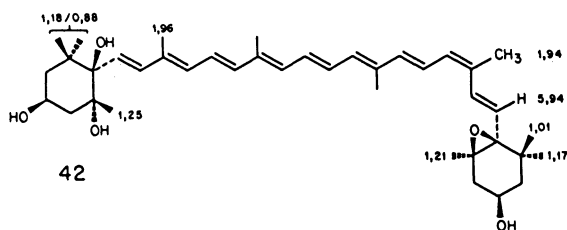


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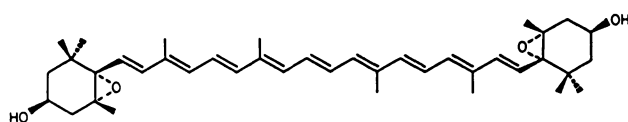
Scheme 16



41



42



24

They all possess triol endgroup A. They differ in the violaxanthin endgroup in 39, and the flavoxanthin- and chrysanthemaxanthin endgroup in 40 and 41. 42 is a (9'Z)-isomer of 39. We have given to them the names latoxanthins (39, 42) and latochromes (40, 41).

In preliminary experiments violaxanthin (24) could be hydrolysed to 40 and 42, so that now also the absolute configurations are ensured.

In finishing I would now like to summarise:

- In general epoxides undergo most varied transformations. In the carotenoid series only few have been meticulously investigated and it would not surprise me if further were discovered.
- Carotenoids with triol endgroups are much more widespread in flowers and fruit than was assumed up to now. They are important for the colouration. But one must also account for them as intermediates in the catabolism of carotenoids to lower molecular cleavage products.

I would like to express my gratitude to the following co-workers for their pleasant co-operation: Andreas Baumeler, Dr. Richard Buchecker, Mrs. Edith Märki-Fischer and Urs Marti.

My thanks also to my Rosa foetida for its dependable bloom, which has presented us with so many new insights.

REFERENCES

1. G. Ohloff and S. Mignat, Liebigs Ann. Chem. 652, 115 (1962).
2. A. Haag and C.H. Eugster, Helv. Chim. Acta 65, 1975 (1982).
3. W. Eschenmoser, P. Uebelhart and C.H. Eugster, Helv. Chim. Acta 65, 353 (1982).
4. W. Eschenmoser and C.H. Eugster, Helv. Chim. Acta 61, 822 (1978).
5. W. Eschenmoser, P. Uebelhart and C.H. Eugster, Helv. Chim. Acta 62, 2534 (1979).
6. W. Eschenmoser, E. Märki-Fischer and C.H. Eugster, Helv. Chim. Acta 67 170 (1984).
7. M. Acemoglu and C.H. Eugster, Helv. Chim. Acta 67, 184 (1984).
8. M. Acemoglu and C.H. Eugster, Helv. Chim. Acta 67, in preparation (1984).
9. M. Acemoglu and C.H. Eugster, Helv. Chim. Acta 67, 471 (1984).
10. P. Karrer and H. Stürzinger, Helv. Chim. Acta, 29, 1829 (1946).
11. D. Goodfellow, G.P. Moss, J. Szabolcs, G. Tóth and B.L.C. Weedon, Tetrahedron Letters 3925 (1973).
12. H. Cadosch, U. Vögeli, P. Rüedi and C.H. Eugster, Helv. Chim. Acta 61, 783, 1511 (1978).
13. W. Eschenmoser and C.H. Eugster, Helv. Chim. Acta, 58, 1722 (1975).
14. H. Cadosch and C.H. Eugster, Helv. Chim. Acta 57, 1466 (1974).
15. E. Märki-Fischer, R. Buchecker, C.H. Eugster, G. Englert, K. Noack and M. Vecchi, Helv. Chim. Acta 65, 2198 (1982).
16. M. Acemoglu, R. Prewo, J.H. Bieri and C.H. Eugster, Helv. Chim. Acta 67, 175 (1984).
17. A.L. Curl and G.F. Bailey, J. Agric. Food. Chem. 2, 685 (1954).
18. J. Gross, M. Carmon, A. Lifshitz and B. Sklarz, Phytochemistry 14, 249 (1975).
19. A. Rüttimann and H. Mayer, Helv. Chim. Acta 63, 1456 (1980).
20. K. Mori, Tetrahedron Letters 2635 (1973).
21. T. Matsuno and S. Sakaguchi, Tetrahedron Letters 24, 911 (1983).
22. R. Buchecker, U. Marti und C.H. Eugster, Helv. Chim. Acta 67, in preparation (1984)
23. R. Buchecker and S. Liaaen-Jensen, Phytochemistry 16, 729 (1977).
24. R. Buchecker, S. Liaaen-Jensen and C.H. Eugster, Phytochemistry 14, 797 (1975).