units in the unit cell, is 2.30, found 2.29 g/cm^{3}.

Bis(benzenetellurenyl) selenide, $(C_6H_5Te)_2Se$. To a solution of 2.5 mmol (1.025 g) diphenylditelluride and 10 mmol (1.44 g) of potassium selenocyanate dissolved in 30 ml of warm methanol was added, under vigorous stirring, 2.5 mmol of bromine dissolved in 2 ml methanol. The solution was stored in a refrigerator for 24 h. The blue-violet crystals of $(C_6H_5Te)_2Se$ were then filtered off. Yield, 1.18 g, or 97 % based on the amount of diphenylditelluride employed. M.p., 64°. (Found: C 30.45; H 2.32; Se 16.42. Calc. for $C_{12}H_{10}SeTe_2$: C 29.50; H. 2.03; Se 16.18).

The crystals are monoclinic, and form long thin needles.

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Loroxanthin from *Chlamydomonas reinhardti*

GEORGE W. FRANCIS,[a] GJERT KNUTSEN[b] and TORLEIV LIEN[b]

[a]*Department of Chemistry and* [b]*Botanical Laboratory, University of Bergen, N-5000 Bergen, Norway*

Previous workers [1-3] have identified the main carotenoid pigments of the green alga *Chlamydomonas reinhardti* as β-carotene, lutein, violaxanthin, trollein and neoxanthin. We have now reinvestigated the carotenoids of *Chl. reinhardti*, strain No. 11-32 (90) from the algal collection of the Institute of Plant Physiology, University of Göttingen, Germany.

Pigments were extracted from the damp-dried algal mass with acetone/methanol (2/1) mixtures, the total extract taken to dryness under reduced pressure and after saponification with methanolic KOH, the carotenoids were separated by thin layer chromatography on Kieselgel G layers with acetone/petrol solvent mixtures. The pigments described by previous workers were readily recognised from their visible light absorption spectra and chromatographic properties. The total pigment content (*ca.* 1.4 mg/g wet weight) and the distribution among the individual pigments were close to those previously reported.

Mass spectrometry showed the expected molecular weights for all polyenes as judged by the observation of molecular ions (M) and ions at M-92 (P), M-106 (Q) and M-158 (T) mass units.[4,5] These ions are formed by the extrusion of 6 or 10 consecutive C-atoms of the conjugated chain with the methyl groups carried by these atoms according to the mechanism of Fig. 1.[6] Treatment with acidified ethanol [7] produced the expected shifts to lower wavelength in the visible light absorption spectra of the epoxides, violaxanthin and neoxanthin. However, while acetylation, with acetic anhydride in pyridine, of lutein, violaxanthin and neoxanthin yielded the required diacetates, the triol previously described as trollein provided a triacetate. Since trollein (*1*) contains only two acetylable hydroxy groups,[8] the identity of the triol with this compound is disproved.

The triol had visible light absorption maxima at 473, 446, (423) nm in acetone and thus had a nonaene chromophore of the type found in lutein (*2*). Mass spectrometry of the triol, in addition to M (584), P (492=M-92), Q (478=M-106) and T (426=M-158) ions, provided an ion at *m/e* 462 (M-122). The mass spectrum of the triacetate (M=710) showed an analogous ion at *m/e* 546 (M-164). The possibility that these latter ions were Q′ ions formed by species in which one of the extruded C-atoms of the chain bore a hydroxymethyl or an acetoxymethyl group, respectively, rather than a methyl group was apparent.[9,10] The partial mass shift of the Q ion, but not of the P ion, was indicative that the substituent was at C-19 rather than at C-20.[10,11]

Further information about the position of the hydroxy groups of the triol was

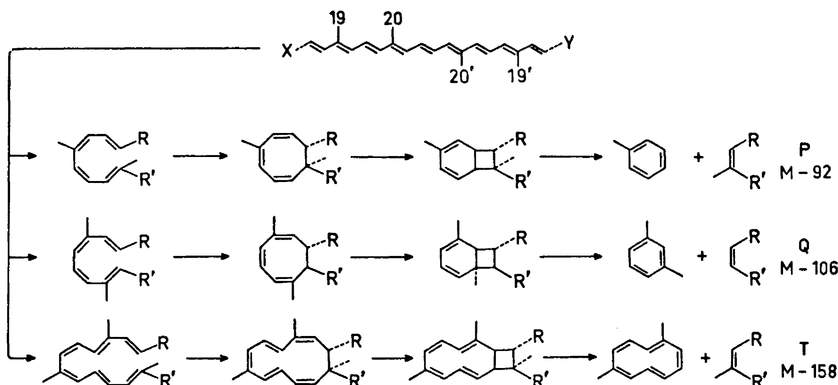
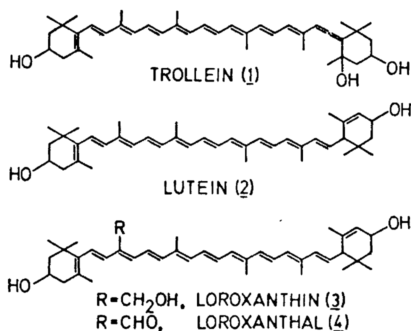


Fig. 1. Mechanism for the formation, by either electron bombardment or prior thermal decomposition, of P (M−92), Q (M−106), and T (M−158) ions observed in the mass spectra of carotenoids.[6]

sought by subjecting it to *p*-chloranil oxidation for 6 h under standard conditions.[12] This reagent is known to effect oxidation of hydroxy groups allylic to the main chromophore, but to leave hydroxy groups allylic to isolated double bonds unaffected. This treatment yielded a single product, somewhat less polar than the starting material, (TLC on Kieselgel G, with 30 % acetone in petrol as eluent, gave the following R_F-values: triol, 0.30; oxidised triol, 0.37; lutein, 0.51) having one absorption maximum only in the visible light region, at 476 nm in acetone and 480 nm in ethanol. Mass spectrometry showed this product to have a molecular weight of 582 and a prominent peak due to loss of 120 mass units at *m/e* 462 was explicable as a Q′ ion. A diacetate of molecular weight 666 could be obtained from this product.



The above evidence shows that only a single oxidisable hydroxy group is allylic to the main chromophore in the triol. Further, the observation of a large shift in the position of the main visible light absorption maximum, 30 nm, on oxidation is believed to be characteristic for carotenoids where one of the normal in-chain methyl groups has been replaced by a hydroxymethyl group.[10,13] Having thus located the oxidised methyl group at C−19 or C−19′, the two remaining hydroxy groups must be placed in the end groups in a way that explains their non-oxidation. The co-occurrence of the triol with lutein in this organism suggests that these hydroxy groups are similarly placed in each case and such an arrangement satisfies the above condition.

Loroxanthin (*3*) is known [10] to occur in a few species of green algae and the data reported for that compound and its *p*-chloranil oxidation product, loroxanthal (*4*), accord with those here reported for the triol and its oxidation product. We thus conclude that the triol, present in *Chlamydomonas reinhardti* and previously identified as trollein (*1*), is in fact loroxanthin (*3*).

When the other xanthophylls present in *Chlamydomonas reinhardti* were subjected to *p*-chloranil oxidation under the same conditions as the triol, no less polar products were formed. However, in the case of the epoxides, prolonged treatment yielded unidentified more polar products,

and since the effects of the epoxy functions in this reaction are unknown the possibility that these compounds contain hydroxymethyl groups cannot be completely ruled out. The weight of available evidence, however, suggests that β-carotene, lutein, violaxanthin, and neoxanthin have been correctly identified by the previous workers.[1-3]

Culturing conditions [14] and chemical methods [15] have been described elsewhere.

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Crystalline Leghemoglobin

XIV. Transfer of Hematin from Lb*a* and Lb*c* to Horse-radish Peroxidase Apoprotein

NILS ELLFOLK, ULLA PERTILÄ and GUNNEL SIEVERS

Department of Biochemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

In 1960 Rossi-Fanelli and Antonini noted the transfer of hematin from *Aplysia* methemoglobin to horse apomyoglobin.[1] Until then the bond between heme and globin had been considered very stable under physiological conditions. A similar migration of hematin has also been shown to take place between some other hemoproteins, *e.g.* myoglobin and horse-radish apoperoxidase (apoHRP).[2] In the present investigation the transfer of hematin from the fast and slow components of soybean leghemoglobin, Lb*a* and Lb*c*, to apoHRP has been studied. The appearance of the peroxidase activity is taken as evidence for the correct binding of hematin to apoHRP. For comparison, the transfer of the hematin of horse myoglobin (Mb I) to apoHRP was also studied.

Materials and methods. Lb*a*, Lb*c* and yeast cytochrome *c* peroxidase (YCCP) were prepared as described previously.[3,4] Horse heart cytochrome *c* was a commercial preparation (Type III) from Sigma Chemical Company (St. Louis, U.S.A.). Horse myoglobin (Mb I) was isolated according to Åkeson and Theorell.[5] Horse radish peroxidase, component C (HRP C) was prepared according to Paul,[6] and its apoprotein according to Theorell and Maehly.[7] The heme-binding capacity of apoHRP was determined by titration with hematin. The peroxidase activity was assayed by the guaiacol method.[8] The concentration of hydrogen peroxide was determined enzymatically according to Yonetani.[9] ApoHRP and leghemoglobin or myoglobin, respectively, were incubated in 0.02 M sodium phosphate buffer, pH 7.0, for 18 h at 25°C. The peroxidatic activity of the solution was measured and the amount of HRP formed calculated.

Results and discussion. Fig. 1 portrays the transfer of hematin from Lb*a*, Lb*c* and Mb I to apoHRP at pH 7.0. The values obtained were corrected for the inherent