units in the unit cell, is 2.30, found 2.29 g/cm². 
Bis(benzentellurenyl) selenide, (C₉H₇Te)₂Se. 
To a solution of 2.5 mmol (1.025 g) diphenyl-
ditelluride 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 meth-
anol. The solution was stored in a refrigerator 
for 24 h. The blue-violet crystals of (C₉H₇Te)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 C₉H₇SeTe₂: C 29.50; H. 
2.03; Se 16.18). 
The crystals are monoclinic, and form long 
thin needles.

13 (1959) 2155.
1274.
3. Haller, W. S. and Irgolic, K. J. J. Organ-
2 (1946) 166.
6. Hauge, S., Opedal, D. and Aarskog, J. Acta 

Received September 24, 1973.

Loroxanthin from 
Chlamydomonas reinhardtii

GEORGE W. FRANCIS,a 
GJERT KNUTSENb and TORLEIV LIENb
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 
algae Chlamydomonas reinhardtii as β-
carotene, lutein, violaxanthin, trolley, 
and neoxanthin. We have now rein-
vestigated the carotenoids of Chl. rein-
hardtii, strain No. 11–32 (90) from the 
algal collection of the Institute of Plant 
Physiology, University of Göttingen, Ger-
many.

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 con-
tent (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 These ions are 
formed by the extrusion of 6 or 10 con-
secutive C-atoms of the conjugated chain 
with the methyl groups carried by these 
atoms according to the mechanism of 
Fig. 1.⁴ Treatment with acetylated ethanol 
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 diaacetates, the triol 
previously described as trolleyin provided a 
triacetate. Since trolleyin (I) contains only 
two acetylbiable hydroxy groups, the iden-
tity 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 spectrom-
etry of the triol, in addition to M (584), 
P (492−M−92), Q (478−M−106) and 
T (428−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 acetoxyethyl 
group, respectively, rather than a methyl 
group was apparent.⁴ 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−20.⁴ ¹¹ 

Further information about the position of the 
hydroxy groups of the triol was
sought by subjecting it to p-chloranil oxidation for 6 h under standard conditions. 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 values: triol, 0.30; oxidised triol, 0.37; futein, 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 606 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. 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 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 reinhardtii and previously identified as trolein (1), is in fact loroxanthin (3).

When the other xanthophylls present in Chlamydomonas reinhardtii 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.

Acta Chem. Scand. 27 (1973) No. 9
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 \( \beta \)-carotene, lutein, violaxanthin, and neoxanthin have been correctly identified by the previous workers.\(^1\)-\(^3\)

Culturing conditions\(^4\) and chemical methods\(^5\) have been described elsewhere.


Received September 24, 1973.

**Crystalline Leghemoglobin**

**XIV. Transfer of Hematin from Lba and Lbc 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, Lba and Lbc, 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.** Lba, Lbc and yeast cytochrome c peroxidase (Y CCP) 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 Yonemani.\(^9\) 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 Lba, Lbc and Mb I to apoHRP at pH 7.0. The values obtained were corrected for the inherent