Carotenoids of *Chlorella Pyrenoidosa*. Pyrenoxanthin, a New Carotenol[1]

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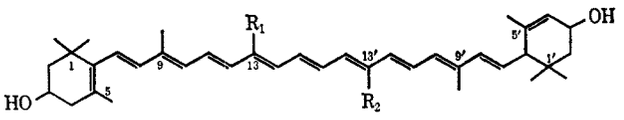
Most of the carotenoids in *C. pyrenoidosa* are identical with leaf pigments of known structures.[2] The new carotenol, pyrenoxanthin, mp 148–149°, is a highly polar pigment which chromatographs ahead of neoxanthin on Micro-Cel C and constitutes 15–25% of the total carotenoids. It is probably identical with a pigment of unknown structure observed previously in *C. pyrenoidosa*[3] and is similar in chromatographic, spectral, and partition characteristics to "trollein" in *Chlamydomonas*.[4] Its visible absorption spectrum (see Experimental Section) was very similar to that of the



1, R_1, $R_2 = CH_3$
2, $R_1 = CH_3$; $R_2 = CH_2OH$ or $R_1 = CH_2OH$; $R_2 = CH_3$

known lutein (1), indicative of a decaene chromophore. The infrared spectrum of pyrenoxanthin showed an OH stretching absorption band at 3450 cm^{-1} and OH deformation or C–O stretching absorption bands in the region 1040–1005 cm^{-1}.

The nmr spectrum of pyrenoxanthin contained six C-methyl resonances which were very similar in values to those recorded for the C-methyl resonances in the two cyclohexene end groups of lutein (1). The signals at τ 8.92 (C-1 geminal methyls, 6 H) and 8.26 (C-5 methyl, 3 H) established the β-ring end group; the signals at τ 9.15 and 9.00 (C-1′ geminal methyls, 3 H each), and 8.39 (C-5′ methyl, 3 H), the other (α-ring) end group. In addition the nmr spectrum indicated the presence of 15 olefinic protons in the τ 3.30–4.80 multiplet, identical with the number present in lutein (1). There were also two in-chain olefinic methyls (τ 8.04, 6 H). The signal at 8.10 (3 H) was indicative of an in-chain–end-of-chain olefinic methyl such as that found in lutein and was accordingly assigned to the C-9′ methyl.

The evidence thus far accounted for nine C-methyl groups. The remaining signal in the nmr spectrum, a singlet at τ 5.46 (2 H), was compatible with a hydroxymethyl group replacing one of the lateral methyl groups in the polyene chain.

Allylic oxidation of pyrenoxanthin with nickel peroxide[5] caused a large bathochromic shift in the visible absorption maxima with loss of fine structure and broadening of the spectrum. The bathochromic effect resulting from this type of cross-conjugation was very similar to that observed in rhodopinol.[6] The oxidized product was reduced to pyrenoxanthin on treatment with $LiAlH_4$. The reduction caused a hypsochromic shift, confirming conjugation of the carbonyl group with the polyene chain in the oxidized product.

The data presented above suggested that the new carotenol pyrenoxanthin represents a derivative of lutein (1) in which a lateral methyl group is oxidized to an alcohol group.

Further information on the structure of pyrenoxanthin was derived from a study of its high resolution mass spectrum (Table I). All peaks greater than 0.1% of

TABLE I
MASS SPECTRAL DATA

Ions	m/e Measured	m/e Calculated	Comment
$C_{40}H_{56}O_3$	584.4235	584.4215	M^+
$C_{40}H_{55}O_2$	567.4192	567.4202	M − OH
$C_{40}H_{54}O_2$	566.4125	566.4110	M − H_2O
$C_{39}H_{51}O_2$	551.3855	551.3876	M − CH_3O, H_2
$C_{40}H_{52}O$	548.4017	548.4005	M − $2H_2O$
$C_{40}H_{50}$	530.3941	530.3900	M − $3H_2O$
$C_{20}H_{27}O_2$	299.2041	299.2004	Smallest fragment containing O_2

base peak were measured with an accuracy of better than 5 ppm and found to be in agreement with structure 2 for pyrenoxanthin. Ions at *m/e* 551, 32, 31, and 30 indicated the presence of a primary OH. This is confirmed further and the position fixed at C-13 (R_1 = CH_2OH; R_2 = CH_3) or C-13′ (R_1 = CH_3; R_2 = CH_2OH) by the fact that *m/e* 299 ($C_{20}H_{27}O_2$) is the smallest fragment with two oxygens. The primary alcohol at C-13 or C-13′ requires at least 18 carbon atoms for a fragment containing two oxygen functions.

Based on the evidences presented, structure 2 is proposed for pyrenoxanthin. However, further degradation and synthesis studies are required to decide on the exact location of the primary alcohol. The structure of 2 suggests that it might have a biosynthetic relationship to 1 and be the first of a series of related natural in-chain hydroxymethyl carotenoids.

Experimental Section[7]

Pyrenoxanthin (2).—The new carotenol was extracted from 500-g wet cells of *C. pyrenoidosa* Chick with methanol and purified by saponification, column chromatography on Micro-Cell C, and crystallization from ethyl ether–petroleum ether (bp 30–60°),

(1) Journal Series No. 1096 of the Hawaii Agricultural Experiment Station.

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(7) Nmr spectra were obtained at 100 MHz and were determined in deuteriochloroform relative to internal tetramethylsilane. High resolution mass spectra were obtained on an AEI Type MS 902 mass spectrometer. The samples were introduced *via* the direct insertion technique at a probe temperature of 180° at 2×10^{-6} mm using PFK as reference compound for the mass measurement.

yielding 30 mg: mp 148–149° (evacuated sealed capillary tube, uncorrected); λ_{max} ($CHCl_3$), mμ ($\epsilon \times 10^{-3}$), 432 sh (73.9), 454 (103), 482 (89.5); λ_{max} (petroleum ether), mμ, 420, 448, 472; ν (KBr pellet) 3450, 1040, 1025, 1005 cm^{-1}; nmr τ 9.15 (s, 3), 9.00 (s, 3), 8.92 (s, 6), 8.39 (s, 3), 8.26 (s, 3), 8.10 (s, 3) 8.04 (s, 6), 5.46 (s, 2), 4.58 (m, 2), 3.68 (m, 13).

Oxidation of Pyrenoxanthin.—Pyrenoxanthin (2 mg) in 5 ml benzene was treated with NiO_2 (30 mg, available oxygen 4.1 $\times$ 10^{-3} g-atom/g of NiO_2 determined by titration) for 60 min.[5] The oxidized product purified by column chromatography on Micro-Cel C exhibited a bathochromic shift in its visible absorption maxima: λ_{max} (petroleum ether), mμ, 450 broad. It was reduced with $LiAlH_4$ in dry ether to a product which was identical by tlc with pyrenoxanthin.

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9-Arylfluorenes. The Energy Barrier for the Inversion of 9-Chloro-9-durylfluorene

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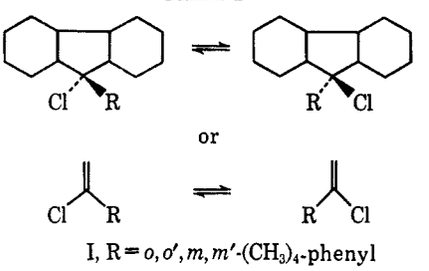
In a recent investigation of the properties of 9-chloro-9-arylfluorenes,[2] it was noted that the presence of substituents in the two *ortho* positions of the aryl group made the nmr spectrum of such a compound temperature dependent, and that this effect was not due to rotation of the aryl group, but rather to a reversible inversion at C-9 in the fluorene nucleus, *e.g.*, to a migration of the chlorine from one side of the fluorene plane to the other (Chart I).[3] Of the various substances investigated, 9-chloro-9-durylfluorene (I) stands out, since its nmr spectrum was the only one which contained more than one temperature-dependent methyl signal. This would permit the calculation of the activation enthalpy and entropy for the inversion of I by a very simple procedure which is based on coalescense parameters only and which was proposed recently by one of us.[5] Herein are reported the results of this study.

CHART I



I, R = o,o',m,m'-$(CH_3)_4$-phenyl
II, R = phenyl

The coalescence parameters of the two collapsing nmr signals of I in $CDCl_3$,[6] which are both due to an exchange of protons between two uncoupled sites, are shown together with the calculated free energy, enthalpy, and entropy of activation ($\Delta F^{\pm}$, $\Delta H^{\pm}$, and $\Delta S^{\pm}$) in Table I. The determination of entropies of activation from nmr data is notorious for the errors involved,[7] and this is compounded in the present case by the fact that the rate is known at only two different temperatures. In order to ensure that our conclusions are meaningful, we have therefore calculated $\Delta F^{\pm}$, $\Delta H^{\pm}$, and $\Delta S^{\pm}$, using the worst combinations of errors. For this we estimate the maximum errors in the experimental data as: $T_c = \pm 1°$; $\delta\nu = \pm 2$ cps.

TABLE I

NMR AND KINETIC PARAMETERS FOR THE INVERSION OF 9-CHLORO-9-DURYLFLUORENE[a]

	Position of CH_3 group	
	o,o'	m,m'
Signal parameters		
T_c, °K	335 ± 1	319 ± 1
$\delta\nu$, cps	113.5 ± 2	18.5 ± 2
k_c, sec^{-1} [b]	244–253 (488–506)	33.6–42.5 (85.0–67.2)
$\Delta F^{\pm}$, kcal/mol[c]	15.52–15.64 (15.06–15.18)	15.87–16.13 (15.44–15.69)
$\Delta H^{\pm}$, kcal/mol[d]	20–30 (20–30)	
$\Delta S^{\pm}$, eu[e]	11–45 (13–46)	

[a] In this table, the customarily calculated energy parameters of inversion are given, together with a set of values (in parentheses) which was calculated with twice the values of k_c as rate. The comparison with energy barriers of solvolytic reactions should be done with these latter values, since in inversions only half of the activated molecules proceed to inverted molecules (and k_c is the measured rate of inversion), whereas in solvolytic reactions all the activated molecules yield solvolysis products. It is interesting to note that energy parameters are remarkably insensitive to the use of k_c and $2 \times k_c$ in the calculation, whereas the main importance is apparently on the value of T_c. [b] $k_c = \frac{\pi}{\sqrt{2}} \times \delta\nu \times \left[1 \times \frac{3\sqrt{2}}{8}\left(\frac{b_E}{\delta\nu}\right) + \frac{21}{64}\left(\frac{b_E}{\delta\nu}\right)^2\right]^{-1}$. See H. H. Schmid, H. Friebolin, S. Kabuss, and R. Mecke, *Spectrochim. Acta*, **22**, 623 (1966); $b_E = 2.5$ cps[2] was used for these calculations. [c] $\Delta F^{\pm} = 1.987 \times T_c \times 2.303 \times (10.035 + \log T_c/k_c)$. [d] $\Delta H^{\pm} = -1.987 \times 2.303 \times (\Delta \log k_c/T_c)/(\Delta 1/T_c)$. [e] $\Delta S^{\pm} = (\Delta H^{\pm} - \Delta F^{\pm})/T_c$.

In spite of the uncertainty in $\Delta S^{\pm}$, the large positive value obtained is clearly meaningful and an indication that the inversion of I in $CDCl_3$ is an unimolecular reaction. We therefore can compare the values in Table I with the known parameters of the energy barrier in the ethanolysis of 9-chloro-9-phenylfluorene (II) in 9:1 ethanol–acetone[8] ($\Delta F^{\pm} = 21.2$ kcal/mol; $\Delta H^{\pm} = 18.1$ kcal/mol; $\Delta S^{\pm} = -11.4$ eu), which is known to have SN1 character.[9]

(1) (a) CIBA Pharmaceutical Co., Summit, N. J. 07901; (b) Bell Telephone Laboratories, Inc., Murray Hill, N. J. 07971; (c) to whom correspondence should be addressed.

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(9) The semiquantitative observation that the solvolysis of II is much slower than that of 9-chloro-9-mesitylfluorene[2] already indicates that $\Delta F^{\pm}$ is lowered by the introduction of two *ortho* methyl groups in the phenyl radical.