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The Structure of Ternstroemiaxanthin, a New Aldehydic C_{40}-Carotenoid*

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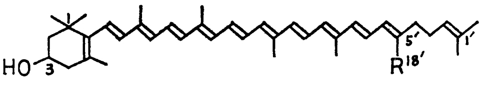
A new carotenoid, ternstroemiaxanthin, was isolated as a main constituent from the red seeds of "*Ternstroemia japonica*," and the structure of the pigment was determined to be 3-hydroxy-β,ψ-caroten-18′-al (I) by spectral and chemical methods.

The deep red coloration of the seeds of "*Ternstroemia japonica*" (Mokkoku) is mainly due to the presence of a new phytocarotenoid, which is named ternstroemiaxanthin. In this paper we will describe its isolation and the structural studies which led to the conclusion that the pigment has the formula (3-hydroxy-β,ψ-caroten-18′-al)[1] represented by I. This is the first example of the presence of aldehydic C_{40}-carotenoid in higher plants.



I R=CHO
II R=CH_2OH

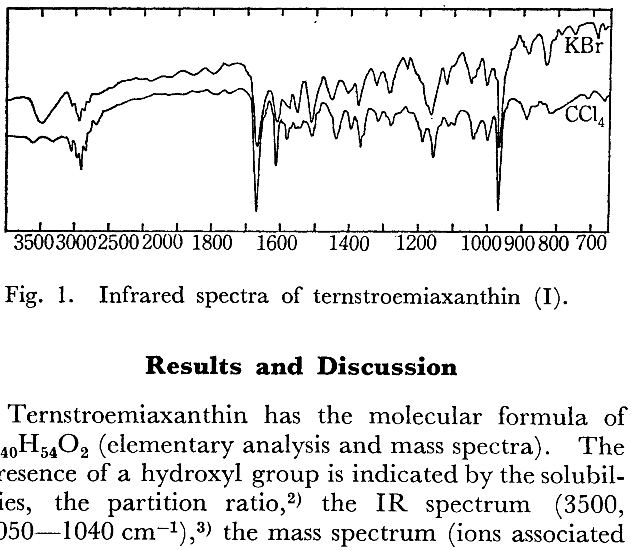


Fig. 1. Infrared spectra of ternstroemiaxanthin (I).

Results and Discussion

Ternstroemiaxanthin has the molecular formula of $C_{40}H_{54}O_2$ (elementary analysis and mass spectra). The presence of a hydroxyl group is indicated by the solubilities, the partition ratio,[2] the IR spectrum (3500, 1050—1040 cm^{-1}),[3] the mass spectrum (ions associated with m/e 18), and the formation of acetate or trimethylsilyl ether. The possibility of a tertiary nature of the group is eliminated by the absence of the characteristic 1150—1130 and 905 cm^{-1} IR absorptions.[4] The possibility of the allylic hydroxyl group can also be excluded by the negative result in the oxidation with chloranil[5] or by the difficulty in an attempted dehydration of the pigment with an acid–chloroform reagent.[6a,b] From the 1042 cm^{-1} position of ν_{C-O} (in carbon tetrachloride), the hydroxyl group seems likely to be secondary and to be on an alicyclic system.[7]

The presence of a formyl group is indicated by the IR [2720 cm^{-1} (in carbon tetrachloride); 1667 (conjugated) and 1160 cm^{-1}] and NMR spectra [δ 9.30]. The conjugated nature of the group can also be seen in the large red shift (10 nm) and the disappearance of fine structures in the UV spectrum taken in a polar solvent.[8]

In *cis,trans*-isomerization with iodine under light,[9] no particular evidence has been obtained to contradict the assumption that the compound has a normal all-*trans* configuration, at least in the central part of the polyene chain. This means that complications due to the presence of thermostable poly-*cis*-,[9] hindered *cis*-,[10] or *cis*-structures with a cross-conjugated aldehyde group[11] can be eliminated in the estimation of the length or the type of chromophore of the pigment from the UV data. A central *cis* or retro-type structure of the chromophore may also be excluded on the basis of the appearance of the δ_{CH} absorption at 965 cm^{-1} (sharp singlet).[12] The pigment absorbs thirteen moles of hydrogen, presumably twelve for double bonds and one for a carbonyl group; it appears, therefore, to be monocyclic. From the λ_{max} of ternstroemiaxanthin (486 nm, petroleum benzin) and its moderately-developed fine structures, it can be inferred that one of the double bonds is out of conjugation and that a part of the chromophore is in a substituted cyclic structure, such as a β-ionone ring, where the effective conjugation of that part with the main chromophore is hindered.[6b] If a partial chromophore involving the terminal carbonyl group is in the cyclic system, however, the fine structures should almost disappear and the λ_{max} should further shift toward blue to a considerable extent, as can be seen in compounds with a 4-keto-β,ψ-carotene chromophore (λ_{max} 462 nm).[13] Therefore, the chromophore given by I, for example, seems appropriate for the pigment. The similarity in the UV spectra of the pigment and of 4′-apo-β,ψ-caroten-4′-al[14] supports this conclusion. The above arguments are also supported by the fact that dihydroternstroemiaxanthin (II) or its diacetate exhibits absorption maxima almost identical with those of β,ψ-carotene,[15] β,ψ-caroten-3-ol[16] or 4′-apo-β,ψ-caroten-4′-ol.[14]

The ions [m/e 413(M−153) and 373(M−193)] in the mass spectrum are considered to give strong evidence for the presence of a hydroxylated β-ionone ring;[17] this is further confirmed by the mass spectra of the trimethylsilyl ether[18] of the pigment and the bistrimethylsilyl ether of the dihydro derivative (II).

The 4-position of the hydroxyl group can be eliminated because of its non-allylic nature. The NMR signal (δ 3.95) of the methine proton adjacent to the hydroxyl group is quite similar to that (δ 4.00) of β,β-carotene-3,3′-diol[19] and different from that (δ 3.55) of β,β-caroten-2-ol.[20] The hydroxyl group can, therefore,

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be assigned to the 3-position on the β-ionone ring.

The ions associated with *m/e* 69 clearly indicate the presence of a isopentenyl group which is common in ψ-type carotenoids.[17] The NMR spectrum, however, shows only nine methyl groups and shows the absence of the δ 1.81 signal which is characteristic of the 5′-methyl group in β,ψ-type carotenoids.[19] This indicates that the formyl group is at the 5′-position in the pigment.

Further confirmations of the structure have been made by chemical means. The lithium aluminium hydride or sodium borohydride reduction of the pigment gave a dihydro compound (II), which gave diacetate or bistrimethylsilyl ether. An attempt to oxidize the aldehyde group into carboxylic acid with silver oxide failed completely. A similar observation regarding the resistance of polyene aldehyde toward oxidation has been previously reported.[21]

Experimental

Anhydrous sodium sulfate was used for drying the solutions. The calcium carbonate or calcium hydroxide used for chromatography was activated by heating it at 150 °C for 3 hr just before use. The instruments used for the measurements of the spectra were as follows: UV spectra, Hitachi, Model EPS-3T. IR spectra, Hitachi, Model 215. NMR spectra, JEOL, Model TS 100. Mass spectra, Hitachi, Model TMS-4, and JEOL, Model JMS-01SG.

Isolation and Properties of Ternstroemiaxanthin (I). The seeds were repeatedly extracted with acetone, the pigment was transferred into petroleum benzin (bp 60—70 °C) by the addition of water, and the solution was concentrated to a deep red oil. The oil was diluted with petroleum benzin and chromatographed on alumina (Merck, neutral; grade II). The ternstroemiaxanthin ester formed a wide violet zone in the middle of the column, besides several minor zones above and below it. A colorless vegetable oil was removed as thoroughly as possible by prolonged washing. The main zone was eluted (petroleum benzin containing a little methanol), and the evaporation residue of the elute was saponified with 10% methanolic potassium hydroxide in petroleum benzin at room temperature for 20 hr. The solution was washed with water, dried, and concentrated to give a reddish-black resinous residue, which was then chromatographed on calcium carbonate from a petroleum benzin solution. A wide, purplish-red main zone (the fourth from the top) was eluted (10% methanolic benzene) and concentrated under reduced pressure. The residue, redissolved in a small amount of methylene chloride, crystallized on the drop-by-drop addition of methanol. Several recrystallizations from the same mixture gave pure ternstroemiaxanthin as blackish-violet needles (8 mg from 1 Kg seeds); mp 160.5 °C (uncorr.). Found: C, 84.32; H, 10.00%. Calcd for $C_{40}H_{54}O_2$: C, 84.75; H, 9.60%. UV spectra: 518(ε 98000), 486(ε 127000), 460_{sh} nm(hexane); 496 nm(ε 115000) (ethanol); 536, 504, 472_{sh} nm(benzene). IR spectra: Fig. 1. NMR spectrum: δ ($CDCl_3$) 1.08(6H, s; 1,1-dimethyl); 1.58 and 1.64(each 3H, s; 1′,1′-dimethyl); 1.73(3H, s, 5-methyl); 1.97(12H, s; 9,13,9′,13′-tetramethyl); 4.00(1H, m, 3-proton); 5.15(1H, m, 2′-proton); 5.98—6.95 (15H, m, polyene chain protons); 9.30(1H, s, aldehyde). Mass spectrum: 566.4132(M, calcd for $C_{40}H_{54}O_2$, 566.4124), 548 (M−18, 2.6%), 497(M−69, 1.1), 487(M−79, 0.8), 479 (M−69−18, 0.6), 474(M−92, 2.5), 460(M−106, 4.5), 456 (M−92−18, 0.7), 442(M−106−18, 1.1), 413(M−153, 1.3), 408(M−158, 1.8), 405 (M−92−69, 1.0), 391 (M−106−69, 1.7), 373(M−193, 1.0), 368(M−106−92, 1.6), 106(32), 91(63), 69(17), 28(100), 18(53).

cis,trans-*Isomerization.* After equilibration in benzene in the presence of iodine and light, the 504-nm maximum of the pigment shifted to 500 nm, accompanied with a decrease in the extinction coefficient, and a *cis*-peak was newly formed at 381 nm, with a shoulder at 361 nm.

Catalytic Hydrogenation:[22] 13.28 mol of hydrogen were absorbed (PtO_2, acetic acid; reference substance, β,β-carotene-3,3′-diol).

The partitioning ratio[2] between petroleum ether (bp 50—60 °C) and 90% methanol was 42 : 58.

The pigment (0.1 mg) dissolved in chloroform (0.2 ml) was treated with three drops of an acid-chloroform reagent[6a] for fifteen minutes. Most of the pigment was recovered unchanged, and no dehydrated product was observed on working up, including a chromatographic examination. The pigment (0.1 mg) was also treated with chloranil (0.09 mg) in a mixture of benzene (0.6 ml) and ethanol (0.07 ml) at room temperature for 20 hr. No product exhibiting wavelength maxima longer than those of the starting pigment was observed in the chromatographic analysis of the mixture.

Acetate. Acetate was prepared by the usual procedure from the pigment and acetic anhydride in pyridine and was purified by chromatography on calcium carbonate. A reddish-black precipitate. Partition ratio: 95.5:4.5 (petroleum benzin and 90% methanol). λ_{max} 518, 486, 457_{sh} nm(petroleum benzin). IR; 2960, 2880, 1738 (CH_3COO), 1670, 1242, 962 cm^{-1}.

Trimethylsilyl Ether. This was prepared from the pigment, hexamethyldisilazane, and trimethylchlorosilane in pyridine,[18] and was purified by chromatography (calcium carbonate, petroleum benzin). Some characteristic peaks in the mass spectrum were as follows: *m/e* 638 [M, for $C_{40}H_{53}O_2Si(CH_3)_3$], 569(M−69), 566(M−72), 548(M−90), 546(M−92), 532(M−106), 480(M−158), 477(M−69−92), 463(M−69−106), 413(M−72−153), 373(M−72−193), 41 (base peak).

Dihydroternstroemiaxanthin (II). Ternstroemiaxanthin (2 mg) dissolved in ethanol (15 ml) was treated with sodium borohydride (50 mg) for thirty minutes. The mixture was then treated as usual, and after chromatographic purification (calcium carbonate, petroleum benzin), a dihydro derivative was obtained as red needles (1.1 mg); mp 174 °C (from benzene-petroleum benzin). Lithium aluminium hydride gave a poorer yield. λ_{max}: 490(ε 107000), 459(ε 122000), 434_{sh} nm(petroleum benzin); 508, 476, 447_{sh} nm(benzene).

Diacetate; λ_{max} 492, 461, 434 nm(petroleum benzin).

Bistrimethylsilyl ether, prepared by the usual procedure,[18] gave the following characteristic peaks: *m/e* 712(M for $C_{46}H_{72}O_2Si_2$), 643(M−69), 622(M−90), 620(M−92), 606 (M−106), 532(M−90−90), 487(M−72−153), 447(M−72−193), 73(base peak).

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