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# A *SECO*-CAROTENOID FROM LEAVES OF TWO CYCADS

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**Key Word Index**—*Ceratozamia kuesteriana*, *C. fuscoviridis*; Zamiaceae cycads; young leaves; carotenoids, 5-hydroxy-5,6-*seco*-β,β-caroten-6-one.

**Abstract**—The young leaflets of *Ceratozamia kuesteriana* and *C. fuscoviridis* show a transitory red-brown coloration due to the presence of a mixture of at least six keto-carotenoids. The major component of this mixture was previously characterized as semi-β-carotenone A new *seco*-carotenoid has now been isolated and identified by its chromatographic and spectroscopic properties as 5-hydroxy-5,6-*seco*-β,β-caroten-6-one.

## INTRODUCTION

There was no information about the carotenoid compositions of photosynthetic tissues of members of the Cycadales, a relict group of ancient gymnosperms still extant, until we recently isolated and characterized semi-β-carotenone (5,6-*seco*-β,β-carotene-5,6-dione, **1**), a carotenoid that has not previously been detected in photosynthetic tissues [1].*

We have examined further the leaves of *Ceratozamia fuscoviridis*, a species provisionally described and named by Moore [3] but later considered by Schuster [4] as one of the forms of *C. mexicana* Brongn. According to Stevenson and Sabato [5] this form is now renamed *C. mexicana* Brongn var. *longifolia* forma fuscoviridis D. Moore ex Schuster. In addition we have also sampled the leaves of *C. kuesteriana* Regel, a species which was recently rediscovered in Mexico [6].

In the first three months or so of growth, the newly formed leaflets of both plants show a very marked red-brown coloration due to the presence of a mixture of keto-carotenoids which, at that stage, constitute *ca* 75–80% of the total carotenoids. The normal chloroplast carotenoids namely β-carotene, lutein, violaxanthin and neoxanthin, constitute the remaining 20–25%. After the characterization of semi-β-carotenone [1] which is the major component of this mixture (*ca* 67% of total carotenoids), our investigation has been extended to some of the other five red components which, although minor, constitute *ca* 11% of the total carotenoids.

Although they are present in the chloroplast, it is likely that the five minor red compounds, together with semi-β-carotenone, are located not in the thylakoid membranes but in very electron-dense bodies of large size which, under the electron microscope, appear similar to plastoglobuli and are found in great numbers in the stroma These pigments are recovered in a surface oily layer when a leaf homogenate is centrifuged.

In a preliminary report, we have presented the first data for three of the minor components [7]. One of these, designated Y, has now been investigated in detail and identified as the novel 5-hydroxy-5,6-*seco*-β,β-caroten-6-one (**2**) by its chromatographic and spectroscopic properties and by chemical correlation with semi-β-carotenone.

## RESULTS AND DISCUSSION

### *Chromatographic properties*

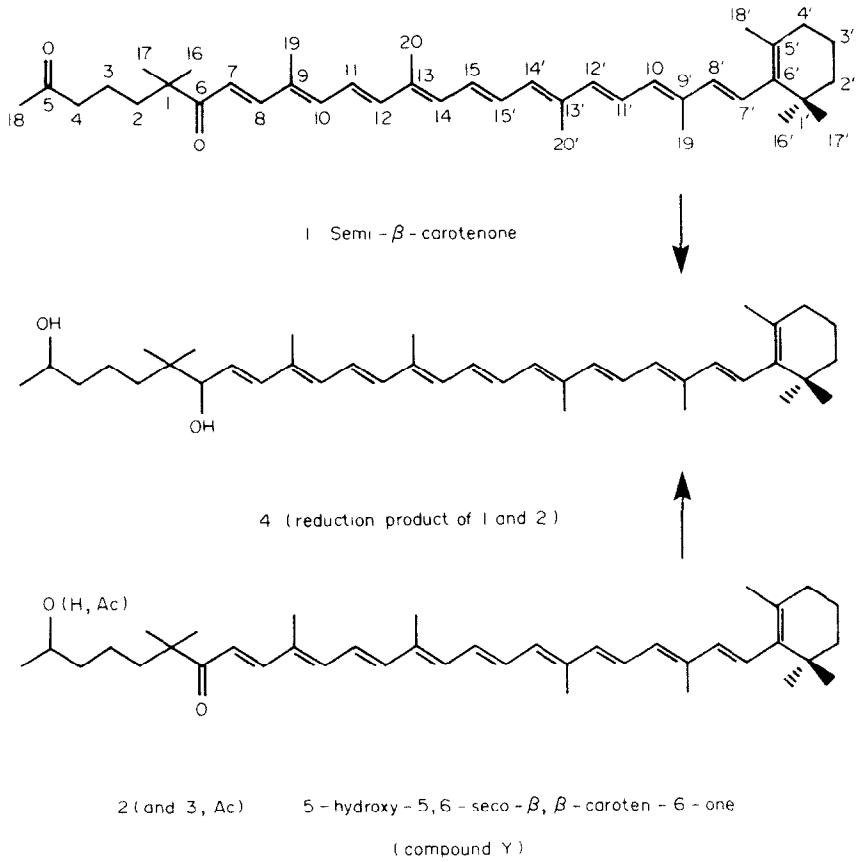
The red compound, Y, was a minor component of the mixture of keto-carotenoids, and comprised *ca* 3% of the total carotenoids. It was isolated by TLC in the system previously used for semi-β-carotenone [1]. Samples from red-brown young leaflets of both *C. kuesteriana* and *C. fuscoviridis* were shown by TLC and HPLC to be identical, and were combined.

An aliquot of a very rapidly prepared extract from a leaf was immediately analysed by reversed-phase HPLC. Six compounds with spectra characteristic of keto-carotenoids, including compound Y, were present along with the normal chloroplast pigments, confirming that Y does occur naturally and is not an artefact produced during storage or TLC. In the reversed phase HPLC system, compound Y chromatographed close to the major component, semi-β-carotenone, but on silica TLC it was substantially more polar than semi-β-carotenone, though less polar than its diol reduction product

### *UV-Vis and IR spectra*

The UV-visible spectra of compound Y and semi-β-carotenone, recorded in five organic solvents, were very similar and showed the rounded shape typical of the presence of a conjugated carbonyl group. In the apolar solvent, *n*-hexane ($\lambda_{max}$ nm: 464 and 486sh) some spectral

*According to the rules for the semi-systematic nomenclature of carotenoids, the *seco* end-group in semi-β-carotenone and its derivatives has priority and is given unprimed numerals [2] In the previous publication [1], the *seco* end-group of semi-β-carotenone was given primed numerals




fine structure was still apparent, in benzene ($\lambda_{max}$ nm: 477) and carbon disulphide ($\lambda_{max}$ nm. 494) very slight shoulders were seen at 498 and 512 nm respectively, but in the more polar solvents ethanol ($\lambda_{max}$ nm 465) and chloroform ($\lambda_{max}$ nm· 478) spectral fine structure was completely absent These data are in agreement with a decaenone chromophore like that of semi-$\beta$-carotenone [1] The IR spectrum of compound Y in $CCl_4$ showed a band at 1670 $cm^{-1}$ attributable to a conjugated carbonyl group

*Chemical reactions and correlations*

Like semi-$\beta$-carotenone and the other minor red compounds, compound Y showed some instability under drastic saponification conditions, but a substantial amount could be recovered unchanged There was no evidence of the formation of a more polar product which would have occurred if compound Y were a carotenoid ester. The acid isomerization test [8] for the presence of a 5,6-epoxy-$\beta$-ring was negative. Under standard acetylation conditions [8], compound Y gave a single, less polar product (**3**) confirming the presence of a primary or secondary hydroxy group

Reduction with sodium borohydride [8] caused a shift in the absorption spectrum (in ethanol) from 465 nm to 420, 443, 471 nm, with a substantial increase in spectral fine structure, showing the presence of a carbonyl group conjugated with the main polyene chain The sodium borohydride reduction product of compound Y was identical (absorption spectrum, co-chromatography on TLC and HPLC) with the reduction product of semi-$\beta$-carotenone, i e. 5,6-*seco*-$\beta$,$\beta$-carotene-5,6-diol (**4**) Compound Y must, therefore, have its two oxygen functions at positions 5 and 6, with the carbonyl group conjugated with the main polyene chain and therefore at C-6, and the hydroxy group at C-5, i e structure **2**.

*Mass spectrometry*

**2** was supported by the comparison of the 70 eV EI mass spectrum of Y with that of semi-$\beta$-carotenone (**1**). The molecular ion peak was at $m/z$ 570 (cf 568 for semi-$\beta$-carotenone) and was consistent with a $C_{40}H_{58}O_2$ formula Analogous significant fragments were also observed In particular, $[M-92]^+$ ($m/z$ 478) and $[M-106]^+$ ($m/z$ 464) were present with an abundance ratio $R=0.16$, a value very close to that of **1**, ($R=0.15$) and in good agreement with the typical low values reported for many monocyclic carotenoids [8]

A significant fragment ion was found at $m/z$ 552 $[M-H_2O]^+$, confirming the presence of a hydroxy-group The most important diagnostic fragmentation was the presence of a fragment ion at $m/z$ 413, $[M-C_9H_{11}O_2]^+$ The same fragmentation, in this case $[M-C_9H_9O_2]^+$, was also present at similar relative intensity in the mass spectrum of semi-$\beta$-carotenone These fragments are ascribed to cleavage of the (C-6)–(C-7) bond, adjacent to the (C-6)-keto-group The molecular fragment (C-1)–(C-6) therefore contains two more hydrogen atoms in compound Y than in semi-$\beta$-carotenone

Reduction of the acetate **3** with lithium aluminium hydride, or of Y itself with sodium borohydride gave the same diol product, the mass spectrum of which showed the molecular ion at *m/z* 572, confirming that one keto group has been reduced. Proof that the reduction products of Y and of semi-β-carotene were identical was obtained by metastable ion (MI) analysis of the molecular ion (*m/z* 572) generated by EI, with a typical MS/MS experiment [10], using the reversed geometry double-focusing VG-MM-ZAB-2F instrument as a tandem mass spectrometer [11]. The MI mass analysed ion kinetic energy (MIKE) spectra from the molecular ions of the two diols were almost superimposable.

As was observed with semi-β-carotenone [1], the intensities ratio of the $[M-92]^+$ and $[M-106]^+$ fragments in the MI-MIKE spectra were reversed with respect to those in the normal EI mass spectra. This can be explained by the observation that the loss of *m*-xylene (106 mass units) from carotenoids occurs as a thermal process as well as a normal electron impact fragmentation [12].

### $^1$H *NMR spectra* (200 *MHz*)

Compound Y is a minor component of the pigment extract of the leaves and could be obtained only in small quantities. Consequently the amount of sample available was small and a $^1$H NMR spectrum of sufficient quality for full analysis and assignment was not obtained. The major characteristic features of the spectrum, however, were consistent with the proposed structure In particular, the methyl group signals were at δ1 98 (C-9,13, 9′ and 13′ methyls), 1.16 (*gem* methyls at C-1′) and 1.69 (methyl at C-5′). No signal was seen at δ2.10 (present in the spectrum of semi-β-carotenone and assigned to the methyl group at C-5) confirming the absence of a C-5 keto-group. Unfortunately, because of lipid impurities in the sample, the C-5 methyl signal for the proposed 5-hydroxy structure, expected at *ca* δ1 25, could not be identified.

The chromatographic and spectroscopic data, in conjunction with the correlation with semi-β-carotenone and their mutual reduction product, the diol **4**, establish the structure of compound Y as 5-hydroxy-5,6-*seco*-β,β-caroten-6-one (**2**) This is the first report of the natural occurrence of a carotenoid with the semi-reduced 5,6-*seco*-β-end-group. The hydroxy-group renders C-5 a chiral centre, but the chirality at C-5 has not been established.

## EXPERIMENTAL

Leaves of *Ceratozamia* species were obtained from the Botanical Gardens of the University of Florence

All general procedures for pigment extraction, TLC separation and purification were conducted as described for semi-β-carotenone [1]. The isolated Y was then chromatographed at least twice on columns of silica gel and the major fraction, eluted with 50% $Et_2O$–petrol, was collected. The sample was finally purified by TLC on silica gel, [pre-washed with $Et_2O$–EtOH (1 1)], in $Et_2O$–petrol (2 3), immediately prior to the determination of its mass spectrum All solvents for this final chromatography and elution were redistilled twice and filtered through a small column of activated alumina immediately before use Reversed-phase HPLC was also employed for the separation, preliminary identification and final purification of carotenoids for $^1$H NMR spectroscopy A Zorbax ODS column 25.0 cm × 4 6 mm (Dupont) was used, with a linear gradient of 0–100% EtOAc in MeCN–$H_2O$ (9·1) at 1 ml/min. *Ca* 180 μg of purified Y was obtained for analysis from 250 g of leaves.

The various chemical tests, e.g. acetylation, saponification, were performed by standard procedures [8], and products analysed by chromatography and co-chromatography on silica gel

The MS (EI, 70 eV, 2 mA) of the compounds were obtained by direct introduction of the samples into the ion source, kept between 200 and 220° without heating the probe and showed the following significant peaks (*m/z*, the respective relative intensities). Compound Y (**2**) 570 (40%); 552 (2 5); 478 (3 2), 464 (20), 413 (3 5); 321 (1 5); 307 (3 5); 69 (100), 45 (50); Y acetylated (**3**) 612 (100%), 520 (15), 506 (80); Y acetylated and reduced with $LiAlH_4$ (**4**) 572 (2%); 554 (5.5), 480 (very weak), 466 (1 5), 462 (1 3); 448 (1 5); 69 (100). Semi-β-carotenone reduced with $NaBH_4$ (**4**) 572 (4%); 554 (42); 480 (very weak), 466 (2), 462 (5), 488 (10); 69 (100)

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