3. Isolation and Absolute Configuration of β,β-Carotene Diepoxide

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The 5,6:5′,6′-diepoxy-5,6:5′,6′-tetrahydro-β,β-carotene, isolated from tubers of a white-fleshed variety of sweet potato (*Ipomoea batatas* LAM.), has been assigned the (5*R*,6*S*,5′*R*,6′*S*)-chirality on the basis of its HPLC, UV/VIS, and CD data.

β,β-Carotene diepoxide has been isolated on several occasions from various sources (see [1]), although always in low yield and never in a crystalline state. In none of these cases has it been shown, whether it exists as a racemate or as a *meso*-form or as a single enantiomer and, if so, of which chirality.

To facilitate the eventual determination of the chirality of this elusive compound, we synthesized some time ago the (5*R*,6*S*,5′*R*,6′*S*)-enantiomer **1** in an unambiguous way [1].

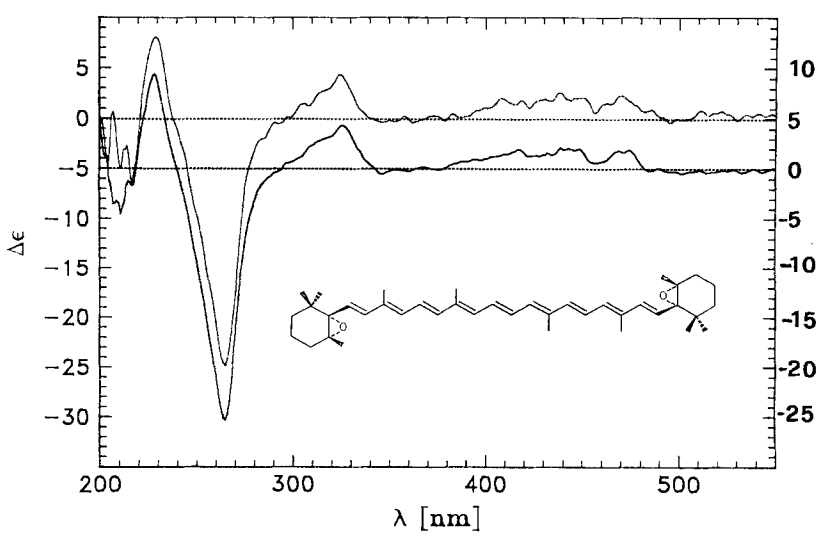

Figure. *CD* (EPA) *of* **1** *from sweet potato* (lower curve, scale at right) *and of synthetic* **1** [1] (upper curve, scale at left)

After our successful isolation and identification of the C(8')-epimeric luteochromes (considered to be transformation products of **1**) from Brazilian sweet potatoes [2], we have now succeeded in obtaining their labile precursor. As described in the *Exper. Part*, after saponification of the carotenoids, chromatography on MgO/*Celite* provided a fast-running main zone which, according to its UV/VIS spectra, colour reactions with acid, and HPLC behaviour, consisted mainly of β,β-carotene diepoxide. Final purification by prep. HPLC afforded nearly pure epoxide with correct spectral data and with CD characteristics very close to those of our synthetic compound (see *Figure*). Therefore, β,β-carotene diepoxide from sweet potatoes is (5*R*,6*S*,5'*R*,6'*S*)-5,6:5',6'-diepoxy-5,6,5',6'-tetrahydro-β,β-carotene (**1**).

It follows that the enzymatic epoxidation of β,β-carotene takes place enantioselectively from the (5*re*,6*si*) side, as it does with the C(3)-hydroxylated xanthophylls, *e.g.* in the conversion of zeaxanthin into antheraxanthin or violaxanthin, and in the one of lutein into lutein epoxide.

Experimental Part

1. *Extraction.* Samples (1 kg) of white-fleshed sweet potatoes were bought at the 'Companhia de Entrepostos e Armazens Gerais de São Paulo'. The exact cultivar is unknown, but the main sorts in this region are IAC 66-118 (Monaliza) and IAC 271 [3]. Fresh sweet potatoes were peeled, cut into small pieces and homogenized with cooled acetone in a waring blendor at moderate speed for three min. After filtration and washing the solids with fresh acetone, the carotenoids were transferred to light petroleum ether by addition of H_2O. After careful washing with H_2O, the carotenoids were isolated and saponified with 10% methanolic KOH at r.t. All steps were carried out under protection against daylight by covering the pigments with dark cloth and/or aluminium foil.

2. *Chromatography.* Separation was achieved on MgO/*Hyflosupercel* 1:2 in 2 × 30 cm glass columns with 3% acetone in light petroleum ether. The first strongly coloured band was eluted and rechromatographed in the same way. Yield 124 μg/100 g fresh flesh. VIS (petroleum ether): 413, 436, 467 nm. Final purification by HPLC on *Spherisorb S-5 CN* with hexane +0,1% $(i\text{-Pr})_2EtN$ (97%)/CH_2Cl_2 + 0,5% MeOH (3%). UV/VIS (qual., Et_2O/isopentane/EtOH 5:5:2 (= EPA)): 264, 412, 435,5, 465,5 nm. CD (EPA, r.t.): *Figure*; estimated $\Delta\varepsilon$ values from UV/VIS data: 343(0), 326(4.43), 294(0), 265(−25.8), 240(0), 229(9.5), 219(0); crystalline synthetic **1**: 341(0), 325(4.4), 295(0), 265(−24.9), 238(0), 229(8.1), 221(0).

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