# 159. Isolation and Characterisation of (5*R*,6*S*,5′*R*,8′*R*)- and (5*R*,6*S*,5′*R*,8′*S*)-Luteochrome from Brazilian Sweet Potatoes (*Ipomoea batatas* LAM.)

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Luteochrome isolated from the tubers of a white-fleshed variety of sweet potato (*Ipomoea batatas* LAM.) has been shown by HPLC, $^1$H-NMR and CD spectra to consist of a mixture of (5*R*,6*S*,5′*R*,8′*R*)- and (5*R*,6*S*,5′*R*,8′*S*)-5,6:5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene (**1** and **2**, resp.). Therefore, its precursor is (5*R*,6*S*,5′*R*,6′*S*)-5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene (**4**). This is the first identification of luteochrome as a naturally occurring carotenoid and, at the same time, gives the first clue to the as yet unknown chirality of the widespread β,β-carotene diepoxide. These facts demonstrate that the enzymic epoxidation of the β-end group occurs from the α-side, irrespective of the presence of OH groups on the ring.

Sweet potato ('batata'; tubers of *Ipomoea batatas* LAM.) is extensively cultivated in tropical and subtropical regions and, especially in its orange-coloured varieties, is a good source of β,β-carotene [1–5]. In São Paulo, Brasil, the most common types are the white-fleshed varieties *Monaliza, Jacareí,* and *Napoleão* with a yearly consumption of 11000–12000 tons [6]. In an earlier analysis of several white-fleshed varieties of sweet potatoes, the following carotenoids were identified: phytofluene, ζ-carotene, neurosporene, β-zeacarotene and β,β-carotene. β,β-Carotene was the main carotene in 6 varieties, β-zeacarotene in 7 varieties, and neurosporene in 2 varieties.

In this investigation of a white-fleshed variety, another main carotenoid **1** was found, not identical with those previously identified. Colour reactions (yellow spots turning green on silica-gel plates, blue coloration in $Et_2O$/HCl) suggested the presence of an epoxide. Comparison of the characteristic UV/VIS and mass spectra with the data of the recently published values of the synthetic isomers of luteochrome [7] showed indeed a very close relationship. Furthermore, based on the $^1$H-NMR chemical shift data and coupling constants, a *trans*-relation of $CH_3$(16) and the polyene chain of **1** was deduced. These conclusions were corroborated by direct comparison with the synthetic luteo-

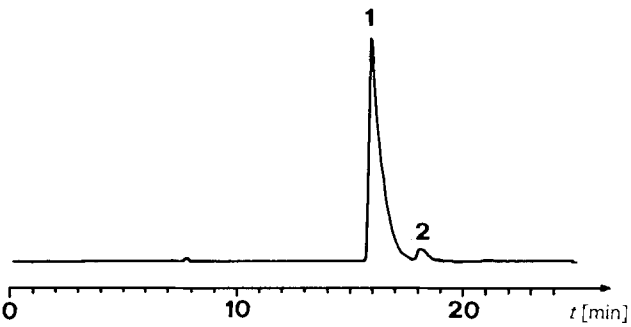
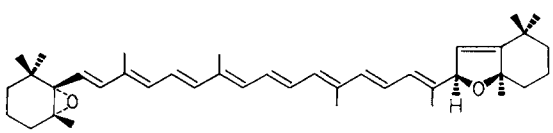



Fig. 1. *HPLC of the luteochrome from sweet potatoes.* Peak *1* with $\lambda_{max}$ 398.5, 421.5, and 448.5 corresponds to **1** and peak *2* with $\lambda_{max}$ 398.5, 421.5, and 448.5 to **2**. Conditions: *Spherisorb S-5 CN* (4.6 × 250 mm) with hexane/Et(i-Pr)$_2$N (1000:1) and $CH_2Cl_2$/MeOH (99.5:0.5) in the ratio of 94:6; 1 ml/min; detection at $\lambda$ 425 nm.

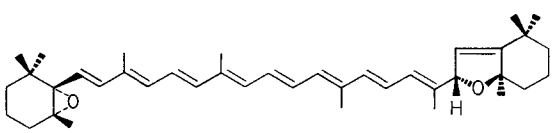
Table. *Selected $^1$H-NMR-Shift Data* (400 MHz, $CDCl_3$) *of* **1**. *A:* **1** from sweet potatoes; *B:* synthetic **1**.

| | $CH_3$(16) | $CH_3$(17) | $CH_3$(18) | CH(7') | CH(8') | $CH_3$(16') | $CH_3$(17') | $CH_3$(18') |
|---|---|---|---|---|---|---|---|---|
| *A* | 0.94 | 1.10 | 1.15 | 5.19 | 5.16 | 1.10 | 1.15 | 1.43 |
| *B* | 0.948 | 1.106 | 1.155 | 5.182 | 5.162 | 1.114[a]) | 1.163[a]) | 1.438 |

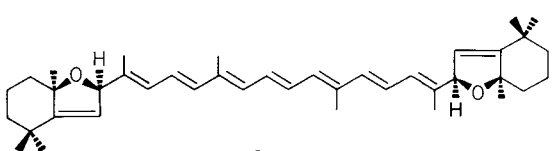
[a]) Interchangeable assignments.



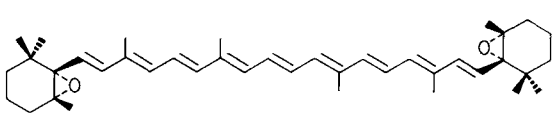
**1**



**2**



**3**



**4**

chromes **1** and **2** by NMR spectra and HPLC, see the *Table* and *Fig. 1*. The HPLC of the natural **1** showed the presence of a minor isomer in a ratio of 1:24. Semipreparative isolation of the main compound **1** and comparison of its CD spectrum with those of compounds of known chirality [7] (see *Fig. 2*) established its identity as (5*R*,6*S*,5′*R*,8′*R*)-luteochrome (**1**).

The minor isomer was shown by cochromatography to be the *cis*-isomer[1]) **2**. Though no CD spectra could be measured, the obvious biogenetic correlation and the well known behaviour of 5,6-epoxides upon rearrangement into 5,8-epoxides leave no doubt concerning the presence of (5*R*,6*S*,5′*R*,8′*S*)-luteochrome (**2**).

A further argument in support of our structure deduction was obtained by the isomerisation of **1** on alumina [8], whereby (5*R*,8*R*,5′*R*,8′*R*)-aurochrome (**3**) was formed with correct UV/VIS and CD properties (see *Fig. 3* (peak 1 corresponds to a (*Z*)-isomer of **4**, see [8]) and *Fig. 4*).

These results not only prove the occurrence of luteochrome in nature for the first time, but even more important, give a clue to the as yet unknown chirality of the widespread diepoxy-β,β-carotene: natural 5,6:5′,6′-diepoxy-5,6,5′,6′-dihydro-β,β-carotene, the precursor of luteochrome **1** in sweet potato must have the (5*R*,6*S*,5′*R*,6′*S*)-chirality (see **4**); synthesis, see [9].

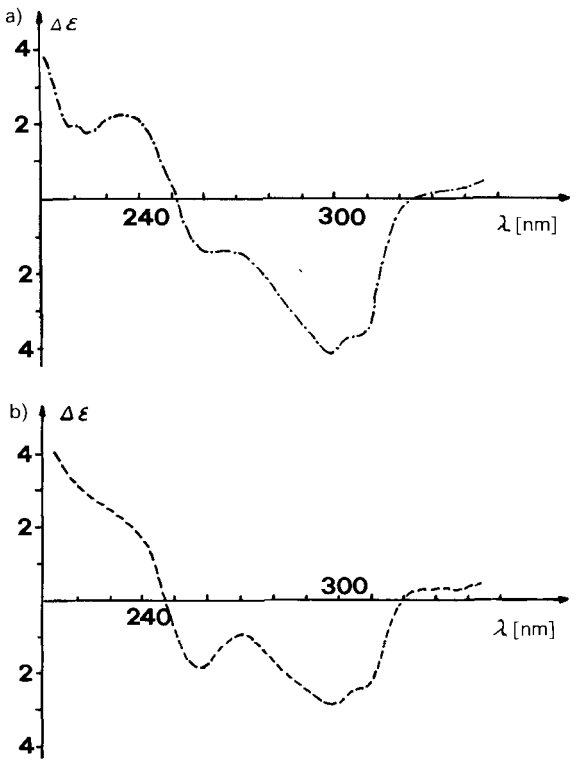



Fig. 2. *CD spectrum* (hexane) a) *of* **1** *from sweet potato* (*E* (251.5) = 0.684) *and* b) *of synthetic* **1** (*E* (251.5) = 0.670)

[1]) The *cis*-configuration refers to the relationship of $CH_3$(18′) and the polyene side-chain on the dihydrofuran ring.

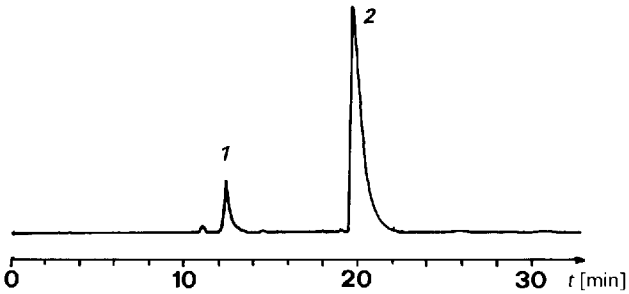



Fig. 3. *HPLC of* **3** *formed from natural* **1** *on* $Al_2O_3$. Peak *1* with $\lambda_{max}$ 375, 395, and 419 has a prominent '*cis*-peak' at 295 nm; peak *2* with $\lambda_{max}$ 379, 401, and 425 corresponds to **3**.

Considering the known chirality of the naturally occurring optically active epoxides, we conclude that the enzymic epoxidation at C(5)/C(6) takes place from the 'α-side', irrespective of the presence of substituents on the ring.

## Experimental Part

1. *Extraction.* Samples of white-fleshed 'batatas' were bought in the main market of São Paulo City (Campanhia de Entrepostos e Armazens Gerais de São Paulo). The exact variety is not known. The peel was immediately removed and the tubers were cut into small pieces, mixed, and blended with cooled acetone in a waring blandor 5 times, with 100 ml of acetone each time. The carotenoids were transferred to light petroleum by addition of $H_2O$, and the extract was washed free of acetone, then dried, evaporated, and saponified overnight at r.t. with 10% KOH/MeOH. After addition of light petroleum, the alkali was removed by thorough washing, and the pigment solution was dried with $Na_2SO_4$ and evaporated.

2. *Isolation of Luteochrome.* The carotenoids were separated by column chromatography on MgO/*HyfloSupercel* 2:1 with 3% acetone in light petroleum. The main band was cut out in the normal manner and eluted with acetone. The eluates were transferred to light petroleum and evaporated.

3. *Identification of the Luteochromes* **1** *and* **2**. Methods, see [7–9]. UV/VIS for **1** (hexane): 399, 422, 449. UV/VIS (EPA): 398.5, 421.5, 448.5. CD (EPA): see *Fig. 2*.

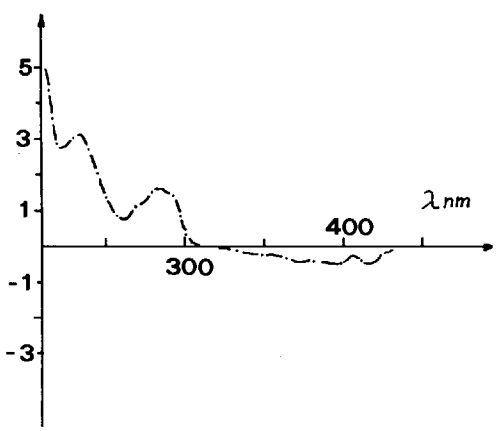



Fig. 4. *CD spectrum* (EPA) *of* **3** (from **1**). $E(398.5) = 1.46$; EPA = $Et_2O$/Isopentane/EtOH 5:5:2.

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