Pergamon

Tetrahedron Letters, Vol. 35, No. 14, pp. 2245-2248, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $6.00+0.00

0040-4039(94)E0245-S

Isolation and Characterisation of Four Allenic (6′*S*)-Isomers of Fucoxanthin

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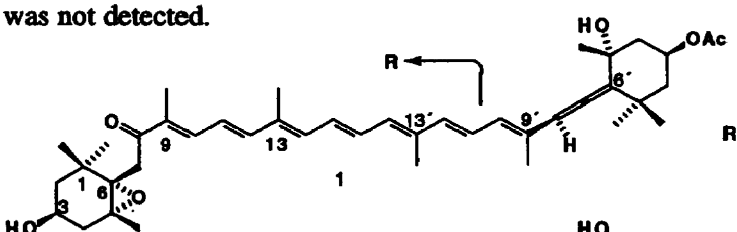
Summary. Four allenic (6′*S*)-isomers: all-*trans* (1), 9′-*cis*, 13′-*cis* and 13-*cis* were obtained by I_2-catalysed stereomutation (strong light, benzene) of natural (6′*R*)-all-*trans* -fucoxanthin; HPLC, VIS, ^{1}H NMR and CD data.

Fucoxanthin (1) is a major carotenoid biosynthesised *de novo* by several algal classes. Its constitution[1-3] and (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-chirality[4] was established in the seventies.

Subsequently the natural occurrence and formation upon iodine catalysed stereomutation of the allenic (6′*S*)-isomer (2) was claimed.[5] This identification was later questioned[6] and disproved.[7-9] It was documented by 2D ^{1}H NMR data that the 9′-mono*cis*-(6′*R*)-isomer (3) of fucoxanthin had been misidentified as the allenic all-*trans* -(6′*S*)-isomer (2). In the iodine catalysed stereomutation mixture studied by Haugan *et al.*[9] the allenic isomer was not detected.



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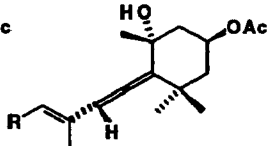

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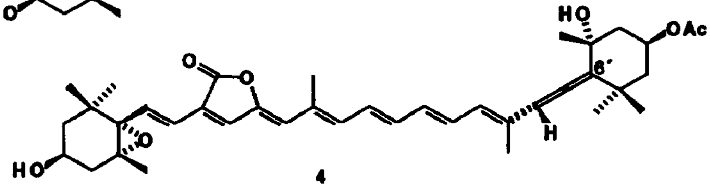

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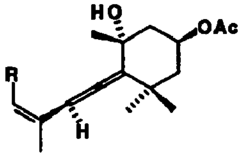

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Recently Yamano *et al.* [10] characterised the (6′*S*)-allenic isomer (4) of peridinin, and Englert *et al.* [11] confirmed the presence as 28% of an iodine catalysed equilibrium mixture by 2D ^{1}H NMR techniques. Total geometrical isomers with (6′*S*, for numbering see[12])-configuration accounted for 45% of the iodine catalyzed equilibrium mixture.[11]

In view of the recent results for peridinin, retrospective considerations lead to the conclusion that < 0.5% of the all-*trans* isomer of (6′*S*)-fucoxanthin (2) according to HPLC and ^{1}H NMR data was present after iodine catalysed stereomutation.[11]

Since we have recently demonstrated[11,12] that the iodine catalysed stereomutation of peridinin is condition dependent and that the geometrical isomerisation can proceed in the absence of allenic isomerisation[11], it was of interest to pursue the isomerisation of fucoxanthin (1) at conditions where the allenic isomerisation of peridinin occurred.

Stereomutation of fucoxanthin (1) was effected in benzene solution in the presence of 2% I_2 in bright September sunshine at 18°C in test tubes of 1 cm diameter at conditions recently employed for geometrical and allenic isomerisation of (6′*R*)-peridinin[11,12] for 2.5h.

HPLC analysis with a system designed for separation of fucoxanthin isomers[9] (5μ silica column with hexane - isopropyl acetate - isopropyl alcohol - *N*-ethyldiisopropylamine 83.9 : 14 : 2 : 0.1, flow 1.5 ml/min), revealed a different isomeric composition than previously obtained in November daylight and larger flasks.

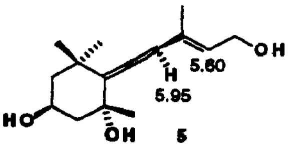
The isomer representing the second largest peak was collected by semipreparative HPLC and identified by VIS, ^{1}H NMR including ^{1}H^{1}H COSY and CD spectroscopy as the allenic all-*trans* -(6′*S*)-isomer (2).

Reversible isomerisation of the all-*trans* -(6′*S*)-isomer (2) in benzene solution in sunlight in the absence of I_2 caused the formation of three *cis* -isomers. These isomers were also present in the iodine catalysed stereomutation mixture obtained from all-*trans* -(6′*R*)-fucoxanthin (1), and were identified after semipreparative HPLC by VIS and ^{1}H NMR spectroscopy in comparison with published data for geometrical isomers of (6′*R*)-fucoxanthin (1)[9] as the mono-*cis* -isomers (6′*S*)-9′-*cis* , (6′*S*)-13′-*cis* and (6′*S*)-13-*cis*. Presumed di*cis* isomers of fucoxanthin represented only a minor peak (<1% of total) by HPLC. Characteristic ^{1}H NMR data (δ-values) for the four (6′*S*)-isomers are given in Table 1.

Table 1. Chemical shifts (δ-values) of the olefinic protons of (6′*S*)-fucoxanthins in comparison with all-*trans* -(6′*R*)-fucoxanthin (1).

Isomer	H-10	H-11	H-12	H-14	H-15	H-15′	H-14′	H-12′	H-11′	H-10′	H-8′
all-*trans* (6′*R*)	7.15	6.58	6.67	6.41	6.64	6.76	6.27	6.35	6.61	6.13	6.06
all-*trans* (6′*S*)	7.15	6.56	6.67	6.41	6.63	6.76	6.27	6.35	6.60	6.14	6.16
9′-*cis* -(6′*S*)	7.15	6.60	6.67	6.41	6.65	6.74	6.27	6.30	6.71	6.05	6.68
13′-*cis* -(6′*S*)	7.15	6.57	6.67	6.41	6.59	6.90	6.14	6.87	6.72	6.19	6.18
13-*cis* -(6′*S*)	7.19	6.60	7.22	6.30	6.82	6.66	6.26	6.35	6.60	6.15	6.16

The chemical shift of the allenic protons in the geometrical isomers of (6′*S*)-fucoxanthin was consistent with that of relevant models, namely isomers of 5-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)-3-methyl-2,4-pentadien-1-ol, synthesised in mixture in a 6 step sequence from actinol.[13]



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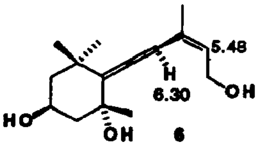

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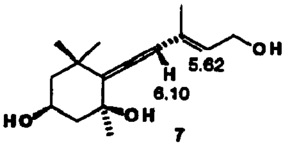

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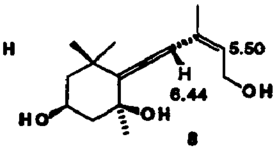

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This is the first unequivocal identification (VIS, ^{1}H NMR, CD) of geometrical *cis*-isomers of (6′*S*)-allenic carotenoids.

Table 2. VIS absorption data for geometrical isomers of (6′*R*) (**1**) and (6′*S*)-fucoxanthin (**2**) in the HPLC eluent.

Isomer	6′*S*		6′*R*	
	λ_{max} nm	%D_B/D_{II}[14]	λ_{max} nm	%D_B/D_{II}[14]
all-*trans*	423, 449, 474	0	422, 449, 474	0
9′-*cis*	322, 330, 419, 445, 472	14	322, 330, 419, 445, 469	11
13′-*cis*	322, 332, 417, 442, 468	47	322, 332, 418, 443, 468	50
13-*cis*	322, 332, 412, 440, 465	45	322, 332, 414, 439, 467	44

After photoisomerisation of all-*trans* -(6′*S*)-fucoxanthin (**2**) in sunlight for 3h no (6′*R*)-isomers were formed, and the proportion of the (6′*S*)-isomers was all-*trans* : 9′-*cis* : 13′-*cis* : 13-*cis* 71 : 20 : 5 : 4, consistent with the quantitative composition of the present iodine catalysed stereomutation mixture: all-*trans* -(6′*S*) : 9′-*cis* -(6′*S*) : 13′-*cis* -(6′*S*) : 13-*cis* -(6′*S*) : all-*trans* -(6′*R*) : 9′-*cis* -(6′*R*) : 13′-*cis* -(6′*R*) : 13-*cis* -(6′*R*) 27 : 11 : 6 : 3 : 35 : 9 : 6 : 4; in total 54% (6′*R*)- and 46% (6′*S*)-isomers, see Fig.1.

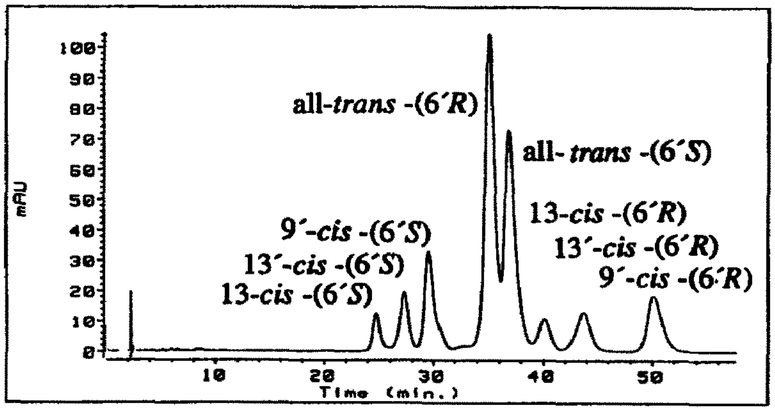

Fig.1. HPLC separation of fucoxanthin stereoisomers.

The all-*trans* -(6′*S*)- and (6′*R*)[9]-isomers showed non-conservative[15], roughly mirror-image CD spectra, reflecting the opposite chirality of the chiral allenic axis in the two diastereomers. The three (6′*S*)-*cis* -isomers exhibited conservative-like CD spectra with similar and opposite Cotton effects relative to all-*trans* -(6′*S*) fucoxanthin (**2**).

No all-*trans* -(6′*S*)-isomer was encountered by HPLC, in the system capable of separating this isomer from (6′*R*)-isomers, in stored solutions of natural (6′*R*)-fucoxanthin.

We have recently demonstrated that all-*trans* -(6′*R*)-fucoxanthin (**1**) is the naturally occurring stereoisomer of fucoxanthin in brown algae.[16] This result has now been confirmed by submitting fresh algal extracts of *Fucus serratus*, *F. vesiculosus* and *Ascophyllum nodosum* to HPLC in the above described HPLC system. No (6′*S*)-isomers could be detected. Hence, no support has been obtained for the suggested biosynthetic precursor role of (6′*S*)-allenic fucoxanthin[5,17] for the naturally occurring (6′*R*)-isomer. The composition of the iodine catalysed quasiequilibrium mixture suggests no significant higher thermodynamic stability of the (6´*R*) over the (6´*S*)-allene (*ca.* 54:46).

In conclusion, it has been demonstrated for fucoxanthin (1), as for peridinin,[10-12] that *R* /*S* isomerisation of the allenic bond (with three sp^2-sp-sp^2 hybridised carbon atoms) may be effected under appropriate light conditions in the presence of iodine, presumably via iodated radicals. The light / surface requirement of this reaction is not yet clear and will be further studied. Geometrical isomerisation of other double bonds (with sp^2-sp^2 hybridised carbon atoms) may be effected, as a separate process, in the absence of iodine. It has been anticipated that photoisomerisation of such carbon-carbon double bonds in carotenoids originates in the S_1 state.[18]

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(*Received in UK* 20 *October* 1993; *revised* 20 *January* 1994; *accepted* 4 *February* 1994)