First preparation and characterization of the allenic carotenoids (all-*E*,6*S*)- and (9′*Z*,6*S*)-neoxanthin

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Conditions for obtaining optimum allenic photoisomerization of (6*R*)-neoxanthin in benzene solution have been studied using diphenyl diselenide, diphenyl ditelluride or iodine as catalyst with various light qualities (sunlight, UVA irradiation or artificial, visible light) and intensities. The effect of adding base in order to prevent furanoid rearrangement was examined. At optimum conditions 77% conversion of (*R*)- to (*S*)-allene was observed for neoxanthin (UVA, 2 × molar excess diphenyl diselenide : neoxanthin, presence of Hünig's base). These conditions were superior to iodine catalyzed isomerization.

(All-*E*,6*S*)- and (9′*Z*,6*S*)-neoxanthin were prepared for the first time and characterized by chromatographic (HPLC) and spectroscopic (VIS, MS, 2D ^{1}H NMR, CD) techniques. Other geometrical isomers of the allenic (6*S*)-series were obtained by (*E*)/(*Z*) isomerization of (6*S*)-neoxanthin and characterized by HPLC and VIS data. At comparable conditions the ease of allenic (*R*)/(*S*) isomerization was peridinin > fucoxanthin > neoxanthin.

Introduction

The naturally occurring (all-*E*,6*R*)-neoxanthin (**1a**, Scheme 1) and (9′*Z*,6*R*)-neoxanthin (**1b**) have been prepared by total synthesis.[1,2] Recently we have characterized other geometrical mono-*Z*- and *Z*,*Z*-isomers of the allenic (6*R*)-series (**1**).[3]

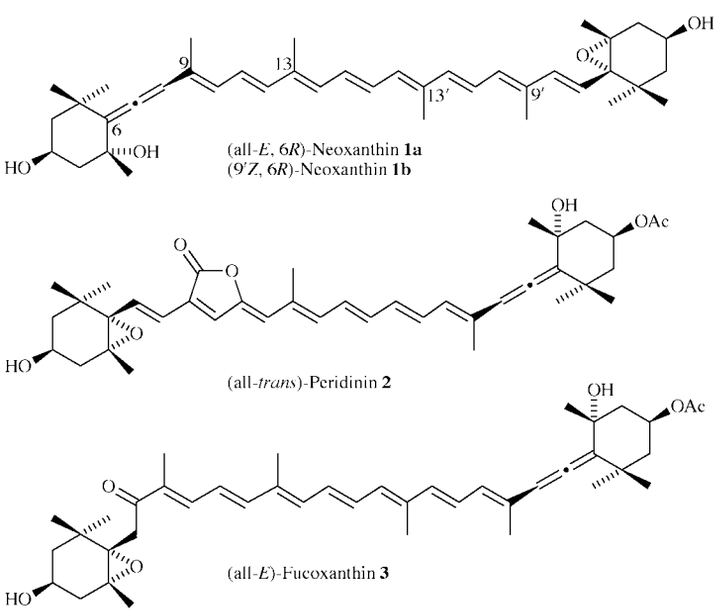
Photoinduced isomerization of the allenic bond in other allenic carotenoids, (6′*R*)-peridinin (**2**) and (6′*R*)-fucoxanthin (**3**), have been achieved using iodine [4–7] or diphenyl diselenide [7,8] as the catalyst.

The possible natural occurrence of (6*S*)-neoxanthin (**4**, Scheme 2) has been questioned.[1,2] We now report the first preparation and characterization of (all-*E*,6*S*)- (**4a**) and (9′*Z*,6*S*)-neoxanthin (**4b**) obtained by photoinduced allenic stereoisomerization. Preliminary results of these studies have already been reported.[9]

Results and discussion

R/*S*-Allenic isomerization

(9′*Z*,6*R*)-Neoxanthin (**1b**), isolated from spinach (*Spinacea oleracea*), was available.[3] Isomerization experiments were carried out in benzene solution using different light qualities (wavelength region) and intensities, with three different catalysts, namely diphenyl diselenide (Ph_2Se_2), diphenyl ditelluride (Ph_2Te_2) or iodine (I_2), at different concentrations, by procedures recently reported.[7,8] Neoxanthin (**1**) readily undergoes



Scheme 1

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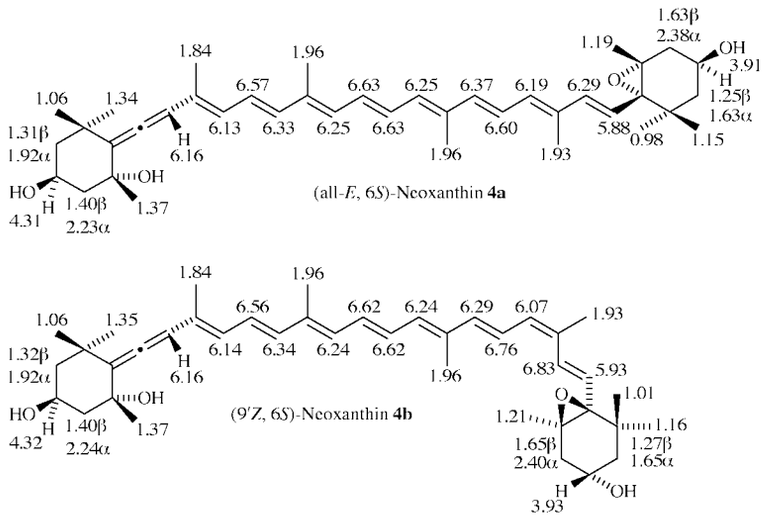

Scheme 2

Table 1 HPLC data for simultaneous separation of mixtures of geometrical isomers of (6*R*)-neoxanthin (**1**) and (6*S*)-neoxanthin (**4**). System 1: Techsphere 5CN (4.6 × 250 mm) column, eluent hexane–dichloromethane–methanol (75.8 : 23.5 : 0.7), detection wavelength 440 nm and flow 1.5 ml min^{-1}

Peak no.	t_R/min	Identification (major isomers)	Reference
1	57.0	(*Z*,*Z*,6*R*)	3
2	57.6	(*Z*,*Z*,6*R*)	3
3	58.0	(*Z*,*Z*,6*S*)	Table 4
4	58.7	(*Z*,*Z*,6*S*)	Table 4
5	59.7	(all-*E*,6*S*) (**4a**) + (9′*Z*,6*S*) (**4b**)	Table 4
6	60.6	(all-*E*,6*R*) (**1a**) + (9′*Z*,6*R*) (**1b**) + (9*Z*,6*S*)	3
7	61.9	(9*Z*,6*R*)	3
8	62.8	(13′*Z* or 13*Z*,6*S*)	Table 4
9	63.7	(13*Z* or 13′*Z*,6*S*)	Table 4
10	64.1	(15*Z*,6*S*)	Table 4
11	64.8	(13′*Z*,6*R*)	3
12	65.6	(13*Z*,6*R*)	3
13	66.1	(15*Z*,6*R*)	3

furanoid rearrangement.[1] Blue-coloured oxonium ions[10] were formed upon exposure of neoxanthin (**1**) to sunshine. Since these reactions are considered to be acid catalyzed,[1,10] the effect of base on the isomerization was investigated.

The isomerizations were monitored by HPLC. The best available HPLC system effected simultaneous separation of geometrical isomers of the allenic (6*R*)- (**1**) and (6*S*)- (**4**) neoxanthin series as presented in Table 1 (*cf.* also ref. 9). (All-*E*,6*R*)-(**1a**) and (9′*Z*,6*R*)-neoxanthin (**1b**) co-eluted, as did the corresponding geometrical isomers (**4a, 4b**) of the allenic (6*S*) series. The integrals of these two peaks, corrected for the presence of the (9*Z*,6*S*)-isomer in the former peak, were used for calculating (6*R*) : (6*S*) ratios. Because isomerization of (6*S*)-neoxanthin (**4a, 4b**) under conditions that only give geometrical isomerization (*cf.* Table 4) provided a quasi-equilibrium mixture with ratios of the individual geometrical isomers similar to that obtained for the allenic (6*R*)-series (**1**),[3] the method described above for calculating the (6*R*) : (6*S*) allenic ratio was justified.

The results of isomerizations carried out in sunlight are given in Table 2. It is concluded that I_2 was not a suitable catalyst combined with sunlight due the inability to reproduce experimental conditions and artifact formation (blue products, presumably oxonium ions).

With 100% Ph_2Se_2 (weight percent relative to carotenoid, corresponding to ~2 : 1 molar ratio) in the presence of Hünig's base a (6*R*) : (6*S*) ratio of 30 : 70 could be achieved with >50% pigment recovery (Table 3). Similar experiments with 100% Ph_2Te_2 as the catalyst gave no allenic isomerization.

The results of isomerization experiments carried out with reproducible, artificial light are compiled in Table 3. Again I_2 was not a suitable catalyst since 2% I_2 caused no allenic isomerization, 20% I_2 caused slight (6*R*) to (6*S*) conversion, while 100% I_2 caused the formation of blue artifacts.

Only geometrical isomerization was achieved with 2% Ph_2Se_2, 20% Ph_2Se_2 gave some allenic isomerization, whereas 100% Ph_2Se_2 at high light intensity resulted in high (6*R*) to (6*S*) conversion. Addition of base prevented furanoid artifact formation, but reduced the rate of allenic isomerization. Optimum (6*R*) to (6*S*) allenic conversion was obtained in UVA irradiation, namely 77% (*S*)-allene with 55% pigment recovery in the presence of base. It is concluded that Ph_2Se_2 is superior to iodine for (*R*)/(*S*) allenic isomerization of the strongly acid sensitive neoxanthin (**1**).

In comparison with the two other allenic carotenoids studied under similar conditions,[5–7] namely peridinin (**2**) and fucoxanthin (**3**), the ease of allenic isomerization decreased in the order peridinin (**2**) > fucoxanthin (**3**) > neoxanthin (**1**). With reference to the radical mechanism proposed for photoinduced diphenyl diselenide mediated allenic isomerization,[7,9] the possibility of electron delocalization of the radical intermediate to the carbonyl functions of peridinin (**2**) and fucoxanthin (**3**) may serve to rationalize this observation.

(All-*E*,6*S*)-Neoxanthin (4a) and (9′*Z*,6*S*)-neoxanthin (4b)

The all-*E* (**4a**) and 9′*Z* (**4b**) geometrical isomers of (6*S*)-neoxanthin (**4**) were isolated after isomerization experiments by preparative HPLC in two subsequent systems. The mixture of all-*E*-and 9′*Z*-isomers obtained by System 1 was separated in System 2.

The all-*E*-isomer (**4a**) was characterized by VIS, MS, 2D ^1H NMR and CD spectroscopy. ^1H NMR assignments are included in Scheme 2, with the expected 0.13 ppm downfield shift of the allenic proton in the (6*S*)- relative to the (6*R*)-series.[4,6] The CD spectrum of (all-*E*,6*R*)-neoxanthin (**1a**) has been reported.[1] It is known that the CD spectrum is mainly dictated by the epoxidic end group with little contribution from the allenic end group.[11] The location of the main maxima and minima in the CD spectrum of the (all-*E*,6*S*)-isomer (**4a**) roughly corresponded to that reported for **1a**.

The 9′*Z*-isomer (**4b**) was characterized by VIS and 2D ^1H NMR data (see ^1H NMR assignments in Scheme 2). Geometrical isomerization of (all-*E*)- and (9′*Z*)-(6*S*)-neoxanthin was carried out under conditions that would not promote allenic isomerization (2% Ph_2Se_2, artificial light, presence of base). The isomeric composition obtained at quasi-equilibrium is given in Table 4.

Table 2 Reaction conditions and results for eleven stereoisomerization experiments of (6*R*)-neoxanthin (**1**) in benzene solution (40 μg ml^{-1}) in sunshine with different catalysts[a]

Base[b]	Catalyst	Date (light intensity/μE m^{-2} s^{-1})	Time/h	Recovery (%)	(6*R*):(6*S*) ratio
—	2% I_2	04.06.97 (2000–2700)	4	89	100:0
—	20% I_2	29.04.97 (2200–2700)	8	84	94:6[c]
—	20% I_2	04.06.97 (2000–2700)	4	71	60:40[c]
—	100% I_2	05.06.97 (2200–2500)	1	—	—[c]
—	20% Ph_2Se_2	18.09.96	4	12	59:41
+	20% Ph_2Se_2	18.09.96	4	68	83:17
+	40% Ph_2Se_2	19.09.96	6	71	73:27
—	100% Ph_2Se_2	23.04.97 (2300–2500)	4	3	100:0
+	100% Ph_2Se_2	28.04.97 (2200–2600)	4	76	61:39
+	100% Ph_2Se_2	02.10.96	3	74	42:58
+	100% Ph_2Te_2	02.10.96	3	96	100:0

[a] 0.10–0.50 mg of **1** was used. [b] *N*-Ethyldiisopropylamine, ~1 vol%. [c] Artifacts formed.

Table 3 Reaction conditions and results for fourteen stereoisomerization experiments of (6*R*)-neoxanthin (**1**) in benzene solution (40 μg ml^{-1}) in darkness or in artificial light without and with different catalysts[a]

Base[b]	Catalyst	Light intensity/μE m^{-2} s^{-1}	Time/h	Recovery (%)	(6*R*):(6*S*) ratio
—	—	3150	8	88	100:0
+	—	3150	8	90	100:0
—	2% I_2	3150	4	89	100:0
—	20% I_2	3150	4	82	94:6[c]
—	100% I_2	3150	1	—	—[c]
—	2% Ph_2Se_2	3150	4	82	100:0
+	2% Ph_2Se_2	3150	4	83	100:0
—	20% Ph_2Se_2	3150	4	70	80:20
+	20% Ph_2Se_2	3150	10	75	78:22
—	100% Ph_2Se_2	500	4	82	100:0
—	100% Ph_2Se_2	3150	12	56	30:70
+	100% Ph_2Se_2	0	6720	86	100:0
+	100% Ph_2Se_2	3150	14	72	46:54
—	100% Ph_2Te_2	3150	8	53	100:0

[a] 0.10–0.16 mg of **1** was used. [b] *N*-Ethyldiisopropylamine, ~1 vol%. [c] Artifacts formed.

Table 4 Retention times HPLC System 1 (Techsphere 5CN (4.6 × 250 mm) column, eluent hexane–dichloromethane–methanol (75.6:23.5:0.9), detection wavelength 440 nm and flow 1.5 ml min^{-1}), VIS data and relative abundance of geometrical isomers at quasi-equilibrium obtained upon stereoisomerization of (6*S*)-neoxanthin (**4**) in benzene with 2% Ph_2Se_2 at 3150 μE m^{-2} s^{-1} for 4 h

					% of total	
t_R/min	λ_{max}/nm (HPLC-eluent)	%III/II[c]	$\%D_B/D_{II}$[c]	Geom. isomer	with base[a]	without base[a]
23.9	331, 410, 432, 460	71	24	*Z*,*Z*	Trace	Trace
24.8	330, 410, 432, 460	65	23	*Z*,*Z*	1	2
25.6	330, 411, 434, 462	76	4	*Z*,*Z*	1	9[b]
26.5	330, 411, 434, 463	64	5	*Z*,*Z*	1	1
28.5	330, 418, 443, 473	89	3	all-*E* + 9′*Z*	73	60
30.3	330, 415, 439, 468	87	5	9*Z*	14	18
31.7	330, 415, 438, 467	62	14	?	2	3
33.4	331, 413, 437, 466	70	37	13′*Z* or 13*Z*	4	4
35.0	331, 413, 437, 466	70	38	13*Z* or 13′*Z*	4	3
35.1	330, 416, 439, 469	67	53	15*Z*	1	1

[a] Presence of *N*-ethyldiisopropylamine, ~1 vol%. [b] Including some artifacts. [c] $\%D_B/D_{II}$ is the *cis*-peak intensity and %III/II is the spectral fine structure as defined in ref. 12.

The identification of the mono-*Z*-isomers was based on λ_{max} shifts of 4–6 nm relative to the all-*E*-isomer (**4a**) and *cis*-peak intensities ($\%D_B/D_{II}$[12]) (*cf.* results for the (6*R*)-series[3]). The four *Z*,*Z*-isomers had λ_{max} shifts of 9–11 nm relative to the all-*E*-isomer (**4a**) and presumably represented the (9*Z*,9′*Z*)-, (9*Z*,13*Z*)-, (9*Z*,13′*Z*)-, (9′*Z*,13*Z*)- or (9′*Z*,13′*Z*)-isomers. The relative polarity of the geometrical (6*S*)-isomers corresponded to that reported for the (6*R*)-series[3] (*cf.* also Table 1).

Reversible (*S*)/(*R*) allenic isomerization of (6*S*)-neoxanthin (**4**) was carried out at high light intensity in the presence of 100% Ph_2Se_2, reaching a similar quasi-equilibrium mixture as obtained when performing the isomerization with the (6*R*)-isomers (**1**) (see Fig. 1). The quasi-equilibria for Ph_2Se_2 (100%) mediated (*R*)/(*S*) allenic photoisomerization of peridinin (**2**), fucoxanthin (**3**) and neoxanthin (**1**) with the same artificial high light intensities contained the same proportion (~75%) of allenic (*S*)-isomers.[7,8] The high yield of allenic (*S*)-isomers has been rationalized on a mechanistic basis.[7] With UVA irradiation a slightly higher proportion (~77%) of (6*S*)-neoxanthin (**4**) was present at quasi-equilibrium (Table 3) consistent with previous findings (~85%) for peridinin (**2**).[7]

In conclusion, it has been demonstrated that the (6*S*)-allenic isomers of neoxanthin (**4**) may be obtained by Ph_2Se_2 mediated photoisomerization in the presence of base (up to 77% yield of the (6*S*)-isomers, 55% pigment recovery) on a semipreparative scale. The availability of (6*S*)-neoxanthin (**4**) may facilitate a further search for (6*S*)-allenic carotenoids in nature (*cf.* refs. 1,3 and 13).

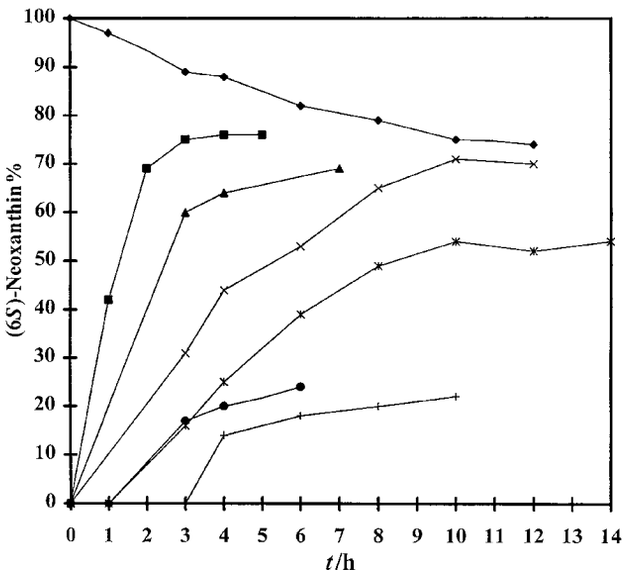

Fig. 1 Allenic isomerization of (6*R*)-neoxanthin (**1**) to (6*S*)-neoxanthin (**4**) as a function of time with diphenyl diselenide as a catlyst at high light intensity using (+) 20% catalyst with base, (●) 20% catalyst without base, (★) 100% catalyst with base, (×) 100% catalyst without base, (▲) 100% catalyst with base in sunlight (25.09.96) and (■) 100% catalyst with base in UVA irradiation. The reversible isomerization of (6*S*)-neoxanthin (**4**) to (6*R*)-neoxanthin (**1**) with 100% catalyst without base is also given (◆).

Experimental

Materials

(9′*Z*,6*R*)-Neoxanthin (**1b**) was isolated from spinach (*Spinacea oleracea*) as previously described[3] and used for the isomerization experiments.

General methods

General precautions for work with carotenoids were taken.[14] Instruments were as previously employed.[15]

Isomerization

Equipment, including light sources, were as specified previously.[7,8] Isomerizations were carried out in benzene (40 μg carotenoid ml^{-1}); the amount (mg) of carotenoid used is given for each experiment. *N*-Ethyldiisopropylamine (Hünig's base) was used as the base (~1 vol%). The isomerizations were monitored by HPLC equipped with a diode array detector. Recoveries were determined spectrophotometrically using the same extinction coefficient at λ_{max}.

Analysis

HPLC System 1: Techsphere 5 CN (anal. column 4.6 × 250 mm, prep. column 10 × 250 mm); hexane–dichloromethane–methanol 75.6:23.5:0.9; detection wavelength 440 nm; flow anal. 1.5 ml min^{-1}, prep. 3.0 ml min^{-1}. HPLC System 2: Ultrasphere Cyano (prep. column 10 × 250 mm); hexane–isopropyl acetate–methanol–*N*-ethyldiisopropylamine (79:20:0.9:0.1); detection wavelength 445 nm; flow 3.0 ml min^{-1}.

(All-*E*,6*S*)-Neoxanthin 4a

Available 0.3 mg (HPLC pure). VIS λ_{max} (ethanol)/nm 418, 443, 469, %III/II[12] = 89; CD λ_{max} (ethanol)/nm (rel. $\Delta\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$) 217 (0), 230 (−1,6), 243 (0.1), 267 (1.6), 282 (0), 295 (0.4), 314 (0); ^{1}H NMR, ^{1}H^{1}H COSY ($CDCl_3$, 400 MHz) δ (ppm) 0.98 (s, 3H, Me-16′), 1.06 (s, 3H, Me-17), 1.15 (s, 3H, Me-17′), 1.19 (s, 3H, Me-18′), 1.25 (m, 1H, H-2′β), 1.31 (m, 1H, H-2β), 1.34 (s, 3H, Me-16), 1.37 (s, 3H, Me-18), 1.40 (m, 1H, H-4β), 1.63 (m, 1H, H-2′α), 1.63 (m, 1H, H-4′β), 1.84 (s, 3H, Me-19), 1.92 (m, 1H, H-2α), 1.93 (s, 3H, Me-19′), 1.96 (s, 6H, Me-20,20′), 2.23 (m, 1H, H-4α), 2.38 (m, 1H, H-4′α), 3.91 (m, 1H, H-3′), 4.31 (m, 1H, H-3), 5.88 (d, $J_{7',8'}$ = 15.5 Hz, 1H, H-7′), 6.13 (d, $J_{10,11}$ = 11.2 Hz, 1H, H-10), 6.16 (s, 1H, H-8), 6.19 (d, $J_{10',11'}$ = 11.4 Hz, 1H, H-10′), 6.25 (m, 2H, H-14,14′), 6.29 (d, $J_{7',8'}$ = 15.4 Hz, 1H, H-8′), 6.33 (d, $J_{11,12}$ = 14.8 Hz, 1H, H-12), 6.37 (d, $J_{11',12'}$ = 14.7 Hz, 1H, H-12′), 6.57 (dd, $J_{11,12}$ = 14.9 Hz, $J_{10,11}$ = 11.9 Hz, 1H, H-11), 6.60 (dd, $J_{11',12'}$ = 15.0 Hz, $J_{10',11'}$ = 12.1 Hz, 1H, H-11′), 6.63 (m, 2H, H-15,15′).

(9′*Z*,6*S*)-Neoxanthin 4b

Available 0.1 mg (HPLC pure). VIS λ_{max} (ethanol)/nm 413, 436, 464, %III/II[12] = 83, $\%D_B/D_{II}$[12] = 3; ^{1}H NMR, ^{1}H^{1}H COSY ($CDCl_3$, 400 MHz) δ (ppm) 1.01 (s, 3H, Me-16′), 1.06 (s, 3H, Me-17), 1.16 (s, 3H, Me-17′), 1.21 (s, 3H, Me-18′), 1.27 (m, 1H, H-2′β), 1.32 (m, 1H, H-2β), 1.35 s (3H, Me-16), 1.37 s (3H, Me-18), 1.40 (m, 1H, H-4β), 1.65 (m, 1H, H-2′α), 1.65 (m, 1H, H-4′β), 1.84 s (3H, Me-19), 1.92 (m, 1H, H-2α), 1.93 (s, 3H, Me-19′), 1.96 (s, 6H, Me-20,20′), 2.24 (m, 1H, H-4α), 2.40 (m, 1H, H-4′α), 3.93 (m, 1H, H-3′), 4.32 (m, 1H, H-3), 5.93 (d, $J_{7',8'}$ = 15.4 Hz, 1H, H-7′), 6.07 (d, $J_{10',11'}$ = 11.0 Hz, 1H, H-10′), 6.14 (d, $J_{10,11}$ = 11.5 Hz, 1H, H-10), 6.16 (s, 1H, H-8), 6.24 ("d", J ~ 8 Hz, 2H, H-14,14′), 6.29 (d, $J_{11',12'}$ = 14.8 Hz, 1H, H-12′), 6.34 (d, $J_{11,12}$ = 15.1 Hz, 1H, H-12), 6.56 (dd, $J_{11,12}$ = 14.7 Hz, $J_{10,11}$ = 11.1 Hz, 1H, H-11), 6.62 ("d", J ~ 11 Hz, 2H, H-15,15′), 6.76 (dd, $J_{11',12'}$ = 14.6 Hz, $J_{10',11'}$ = 11.7 Hz, 1H, H-11′), 6.83 (d, $J_{7',8'}$ = 15.4 Hz, 1H, H-8′).

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