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Algal Carotenoids. IX.* Absolute Configuration of β,ε-Caroten-2-ol, β,β-Caroten-2-ol, and β,β-Carotene-2,2′-diol

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The previous tentative stereochemical assignments 2*R*,6′*R* for β,ε-caroten-2-ol (*1a*), 2*R* for β,β-caroten-2-ol (*2a*), and 2*R*,2′*R* for β,β-carotene-2,2′-diol (*3*) have been confirmed.

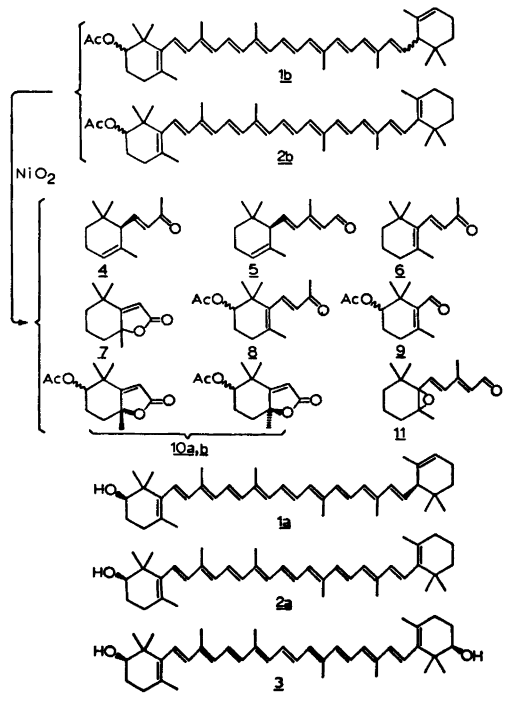
Oxidative (NiO_2) degradation of a mixture of β,ε-caroten-2-yl acetate (*1b*) and β,β-caroten-2-yl acetate (*2b*) gave *ca.* 50 components (GLC), among which (+)-α-ionone (*4*), the ε-apo-carotenal *5*, β-ionone (*6*), dihydroactinidiolide (*7*), 2-acetoxy-β-ionone (*8*), 2-acetoxy-β-cyclocitral (*9*), the *cis-trans* isomeric acetylated lactones *10a,b* and presumably the artefact epoxide *11* were identified from GLC, UV, MS, and CD data.

Whereas isolation of (+)-α-ionone (*4*) defined the 6′*R* configuration of *1a*, the 2*R* configuration followed from application of the modified Horeau method to *2a* and previous CD correlations.

For convenience carotenoid numeration has been used.

Recently we have isolated the first carotenoids possessing 2-hydroxylated β-rings, namely β,ε-caroten-2-ol (*1a*), β,β-caroten-2-ol (*2a*) and β,β-carotene-2,2′-diol(*3*) from the green alga *Trentepohlia iolithus* (L.) Wallroth.[1] Whereas complete spectral characterization defined the constitutions involved, the chemical reactions of these carotenoids have subsequently been studied.[2]

As to their absolute configurations the fact that β,β-carotene-2,2′-diol (*3*) and zeaxanthin [= (3*R*,3′*R*)-β,β-carotene-3,3′-diol] exhibited very similar CD curves of opposite sign was rationalized employing Mills' rule.[3] Regarding both *3* and zeaxanthin as 4-hydroxylated cyclohexene derivatives with preferred pseudo-equatorial hydroxy groups, 2*R* and 3*R* configuration, respectively, represent opposite half-chair conformations with opposite optical rotation. 2*R*-Stereochemistry for the mono-ol *2a* then followed from CD correlation. Moreover, by using the additivity hypothesis of Klyne and co-



Scheme 1.

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workers [4] 2*R*,6′*R* stereochemistry was inferred for *1a*; see Scheme 1.

Since the application of Mills' rule may lead to erroneous results,[5] it was necessary to check the validity of Mills' rule in this case. Furthermore, independent confirmation of the 6′*R* stereochemistry of *1a* was desirable.

RESULTS AND DISCUSSION

Since β,ε-caroten-2-ol (*1a*) and β,β-caroten-2-ol (*2a*) can only be separated with difficulty,[1] oxidative degradation of a mixture of *1a* and *2a* was effected in the Swiss laboratory by the procedure worked out for lutein (= β,ε-carotene-3,3′-diol).[6]

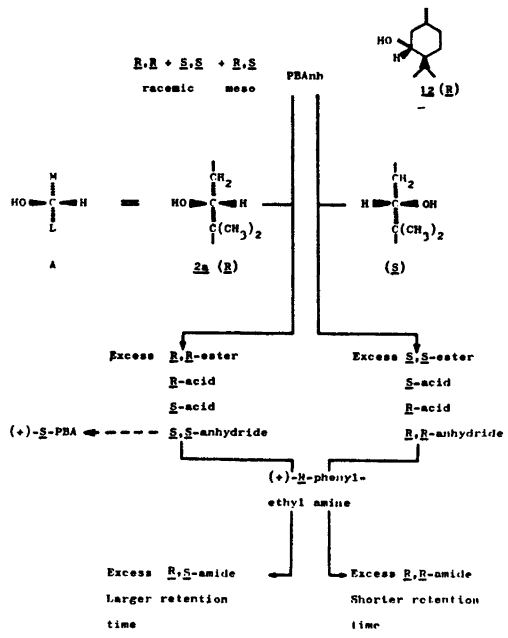
The hydroxy groups were protected by acetylation and a *ca.* 1:1 mixture of semicrystalline *1b* and *2b*, Scheme 1, was oxidized with NiO_2 to give approximately 50 components by GLC. By preparative GLC and subsequent UV, MS, and CD characterization, the following compounds were identified:

(+)-(6*R*)-α-Ionone (*4*) with predicted GLC, UV, and MS properties had opposite Cotton effect to that of (−)-α-ionone (6*S*).[6,7] The ε-apo-11-carotenal (*5*) had GLC, UV,[8] and MS properties consistent with structure *5*. 6*R*-Stereochemistry followed from positive CD 298 nm (curve conform to but bathochromically shifted relative to that of *4*).

β-Ionone (*6*), inseparable from authentic β-ionone, had MS properties as predicted. Dihydroactinidiolide (*7*) [9] with GLC, UV, and MS data consistent with this assignment, was according to the CD data, a racemic mixture. Both *6* and *7* are considered derived from the unsubstituted β-rings and offer no stereochemical information. The same is true for a minor component, from MS alone tentatively identified as the epoxide *11* and considered to be an artefact caused by the oxidation reagent.

Three compounds derived from the 2-acetylated β-end groups were isolated, namely 2-acetoxy-β-ionone (*8*), 2-acetoxy-β-cyclocitral (*9*), and a presumed *cis-trans* isomeric mixture of the acetylated lactones *10a,b*. Again the identifications were based on GLC, UV, and MS data. The CD spectrum of 2-acetoxy-β-cyclocitral (*9*) was analogous to and hypsochromically displaced by *ca.* 20 nm relative to that of the analogue *8* (both resembling that of *2a* itself). Were comparison of the CD spectra of *8* and of 2-acetoxy-β-ionone of known configuration possible, stereochemical conclusions could be made on this basis.

However, evidence for the 2*R* configuration for *2a* was obtained independently using the modified Horeau method.[10] Briefly the Horeau method [11–14] is based on the partial resolution of racemic and meso α-phenylbutyric anhydride (PBAnh) by means of an optically active alcohol, which reacts preferentially with one diastereomer, followed by hydrolysis and optical rotation measurement of the unreacted α-phenylbutyric acid (PBA), Scheme 2.



Scheme 2.

When the isolated PBA is dextrorotatory the alcohol in concern has configuration A; L(arge) and M(edium) refer to the space requirements of the substituents. In the modified version,[10] Scheme 2, the excess enantiomer of the PBAnh is reacted with an optically active primary amine [(+)-*R*-α-phenylethyl amine] followed by quantitative separation of the diastereomeric amides by GLC. Since the chiral amine does not react equally fast with the two enantiomeric PBAnh, parallel experiments with an achiral secondary alcohol like cyclohexanol must be performed.

In the original modification Brooks and Gilbert [10] used a different column for the GLC separation. The relative retention time for the two diastereomeric amides were therefore checked with (−)-*R*-menthol (*12*), corresponding to configuration A, Scheme 2, and consequently providing excess *R,S*-amide.

Three separate experiments with *β,β*-caroten-2-ol (*2a*) each resulted in excess formation of the *R,S*-amide using achiral cyclohexanol in the reference reaction. Lower optical yields in the experiments with *β,β*-caroten-2-ol (*2a*) than for (−)-*R*-menthol (*12*) are explained by the lower solubility of *2a*.

Configuration A, corresponding to *β,β*-caroten-2*R*-ol (*2a*) is thus proved in agreement with the previous tentative assignment.[1] 2*R*-Configuration for *3a* and *1a* follows from the CD correlations already made.[1]

Furthermore it follows from this independent determination of the absolute configuration of *β,β*-caroten-2-ol (*2a*) that 2-acetoxy-*β*-ionone (*8*), 2-acetoxy-*β*-cyclocitral (*9*), and the diastereomeric lactones *10a,b* (Scheme 1) all have the 2*R* configuration.

The fact that the 6′*R* configuration of *1a* corresponds to that of *ε*-rings in related carotenoids isolated from higher plants, suggests a common enzymatic cyclization mechanism.

The implications of the 2*R* stereochemistry here established for the 2-hydroxy-*β*-type carotenoids for the stereochemistry of 2-isopentenyl substituted carotenoids (C_{50}) will be treated elsewhere.[15]

EXPERIMENTAL PART

Materials. Mixed crystalline mono-ols (*1a* and *2a*) were obtained from *Trentepohlia iolithus* after chromatography of the saponified mono-ester fraction as described previously.[1]

Crystalline *β,β*-caroten-2-ol (*2a*) was obtained after chromatography of the mixed mono-ols on magnesia.[1]

Solvents used were of analytical grade.

Instruments. UV spectra (ethanol, if not stated otherwise) were recorded on a Beckman Acta III spectrometer and CD spectra (ethanol, if not stated otherwise) on a Roussel-Jouan Dichrographe model 185 with a xenon high pressure lamp. Due to the small samples these spectra are qualitative only.

Mass spectra were obtained on a GLC/MS Varian MAT CH5 instrument.

Preparative GLC was effected on a Perkin Elmer 900 gas chromatograph with packed glass column 2.5 m/3 mm type SP 1000 (4 % Carbowax 20 M, treated with *p*-nitroterephthalic acid on Chromosorb 100 − 120 mesh) programmed at 160 − 240°C, 5°/min, He-flow 65 ml/min.

Retention times cited below were for an analytical glass capillary column Ucon HB 5100 + H_3PO_4 20 m/0.32 mm, programmed from 80 to 180°C, 2°/min, 0.4 atm. pressure.

For the Horeau experiments a glass capillary column OV 101 + FFAP 23 m/0.3 mm, isotherm, 160°C, 0.5 atm. pressure was employed.

Oxidative degradation

Acetylation. 1a and *2a* (140 mg, 43:57) in pyridine (25 ml) was acetylated quantitatively with acetic anhydride (5 ml) for 24 h at room temperature; yield 139 mg (90 %) *1b* and *2b*.

Degradation. The mixed acetates (*1b* and *2b*, 139 mg) dissolved in ether-benzene (1:1, 100 ml) and NiO_2[16] (8 g) were stirred mechanically at room temperature until decolouration occurred (36 h). The filtrate was concentrated to dryness; yield 28 mg comprising *ca.* 50 components by analytical GLC.

Retention times by analytical GLC were: *α*-ionone (*4*) 10.9 min, *β*-ionone (*6*) 14.3 min, 2-acetoxy-*β*-cyclocitral (*9*) 23.1 min, dihydroactinidiolide (*7*) 25.3 min, *ε*-apo-11-carotenal (*5*) 29.3 min, 2-acetoxy-*β*-ionone (*8*) 37.6 min, and the isomeric lactones *10a,b* 46.8 and 47.1 min.

(+)-(6R)-α-Ionone (4), yield *ca.* 0.3 mg, inseparable from authentic *α*-ionone by co-chromatography, had *m/e* 192 (M), 177 (M − CH_3), 43 (CH_3CO); UV λ_{max} 227 nm; CD (hexane, nm, relative intensities) 368 (−1.0), 352 (−3.5), 337 (−5.0), 324 (−5.3), 315 (−4.0), 244 (+43.5).

(−)-*α*-Ionone (6*S*) for comparison had CD (methylcyclohexane: isopentane 1:3, nm, $\Delta\varepsilon$) 372 (+0.19), 353 (+0.61), 338 (+0.92), 325 (+0.93), 312 (+0.71), in alcohol also 243 (−17.33).

ε-Apo-11-carotenal (*5*) had *m/e* 218 (M); UV λ_{max} 286 nm, reported [8] 286 nm in ethanol: CD (nm, strong) 298 (+), 273 (+), 205 (end absorption +).

β-Ionone (*6*), inseparable from authentic *β*-ionone, had *m/e* 192 (M), 177 (M − CH_3), 43 (CH_3CO).

Dihydroactinidiolide (*7*) had *m/e* 180 (M), 165 (M − CH_3); UV λ_{max} 225 nm, reported [9] 241 nm, solvent unknown; no CD (racemic).

2-Acetoxy-β-ionone (*8*) had *m/e* 250 (M), 235 (M − CH_3), 190 (M − AcOH), 175 (M − CH_3 − AcOH), 43 (CH_3CO); UV λ_{max} 286 nm, reported [17] for *β*-ionone (*6*) 295 nm in ethanol; CD (nm, weak) 370 − 320 (+), 290 − 260 (−), 250 (0), 240 − 200 (end absorption +).

2-Acetoxy-β-cyclocitral (*9*) had *m/e* 210 (M), 195 (M − CH_3), 150 (M − AcOH); UV λ_{max} 243 nm, reported [18] for *β*-cyclocitral 247.5 nm in ethanol; CD (nm) *ca.* 363 (−), 342 (0), *ca.* 315

(+), 282 (0), 250–240 (–), 233 (0), 200 (end absorption +).

Isomeric lactones 10a,b had *m/e* 238 (M), 178 (M–AcOH), 43 (CH_3CO); UV λ_{max} 218 nm, *cf. 7*.

Epoxidic aldehyde 11 had *m/e* 234 (M), 219 (M–CH_3), 191 (M–CH_3–CO→oxepinium ion, $m^*=156$).

Modified Horeau experiments

Procedure by Brooks and Gilbert [10] was used directly for (–)-menthol (*12*) and the first experiment with β,β-caroten-2-ol (*2a*); in the latter case in a heterogeneous system due to low solubility.

α-Phenylbutyric anhydride was prepared as described by others.[19,20]

(–)-R-*Menthol* [10] (*12*). The retention time found for *R*-α-phenylethyl- *R*-α-phenylbutyramide was 7.0 min and for *R*-α-phenylethyl-*S*-α-phenylbutyramide 8.1 min. Standard reaction with cyclohexanol gave the ratio *R,S*-amide:*R,R*-amide = 1:(1.115 ± 0.007). Reaction with *12* gave ratio *R,S*-amide:*R,R*-amide = 1:(0.866 ± 0.015); corrected value by comparison with standard reaction 1:0.866/1.115 = 1:0.775.

β,β-Caroten-2-ol (*2a*). (i) *2a* (4.8 mg) in pyridine (14 μl) and PBAnh (6 μl) was heated in a sealed Pyrex tube at 40–45°C for 1 3/4 h. (+)-*R*-α-phenylethyl amine (6 μl) was added at room temperature, the mixture shaken for 15 min and transferred to ethyl acetate (3–4 ml) for GLC.

Found for standard reaction with cyclohexanol (1 μl = 1 mg) ratio *R,S*-amide:*R,R*-amide 1:(1.115 ± 0.007).

Found for *2a* ratio *R,S*-amide:*R,R*-amide 1:(0.95 ± 0.02); corrected ratio 1:0.85.

(ii) Procedure as for (i) but using *2a* (2.9 mg), pyridine (21 μl, all carotenoid dissolved) PBAnh (3.6 μl) and (+)-*R*-α-phenylethyl amine (3.6 μl).

Found for standard reaction with cyclohexanol (0.6 μl) ratio *R,S*-amide:*R,R*-amide 1:(1.085 ± 0.01).

Found for *2a* ratio *R,S*-amide:*R,R*-amide 1:(0.945 ± 0.007); corrected ratio 1:0.87.

(iii) Procedure as for (i) but using *2a* (2.4 mg), pyridine (14 μl, all carotenoid dissolved), PBAnh (3 μl) and (+)-*R*-α-phenylethyl amine (3 μl).

Found for standard reaction with cyclohexanol (0.5 μl) ratio *R,S*-amide:*R,R*-amide = 1:(1.120 ± 0.011).

Found for *2a* ratio *R,S*-amide:*R,R*-amide = 1:(0.928 ± 0.008); corrected ratio 1:0.83.

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