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OCCURRENCE AND CHIRALITY OF OSCILLAXANTHIN*

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Key Word Index—*Oscillatoria rubescens*; *O. agardhii*; Cyanophyceae; carotenoid composition; oscillaxanthin; (2*R*,2′*R*)-oscillol-2,2′-dirhamnoside; chirality; CD correlation; biological context.

Abstract—The quantitative carotenoid composition of natural blooms of *Oscillatoria rubescens* and *O. agardhii* is reported and compared with previous isolations. Chemical or enzymatic conversion of oscillaxanthin to the chiral aglycone failed. CD-correlation of oscillaxanthin hexaacetate with (2*S*,2′*S*)-bacterioruberin, (2′*R*)-plectaniaxanthin and (2′*R*)-plectaniaxanthin-2′-β-D-glucoside tetraacetate support the 2*R*,2′*R*-configuration for oscillaxanthin.

INTRODUCTION

The phylogenetic relationships and ecological properties of species within the genus *Oscillatoria* are still only vaguely understood. Among the species of *Oscillatoria* capable of building up dense planktonic populations are some strains with red-coloured trichomes. They have unique physiological and ecological interest in their response to the natural environment, by maintaining a prominent overwintering population in some Norwegian lakes.

The quantitative carotenoid composition of two overwintering *Oscillatoria* sp. is reported here. Oscillaxanthin is a carotenoid with occurrence restricted to certain blue-green algae [1–3]. From chemical and spectroscopic evidence oscillaxanthin was assigned the structure oscillol-2,2′-dirhamnoside (**1**, Scheme 1) [4, 5]. Whereas ^{1}H NMR data for its hexaacetate support 1C(L) conformation for the rhamnose moiety [6], assignment of β-L configuration must be tentatively considered [4, 6]. The chirality at C-2,2′ has not previously been solved. We now report the absolute configuration of oscillaxanthin at C-2,2′.

RESULTS AND DISCUSSION

The samples of cyanophytes investigated in this study were collected from overwintering populations under ice in Lake Steinsfjord and Lake Kolbotnvatn. The natural blooms consisted of *Oscillatoria rubescens* DC. var. and *Oscillatoria agardhii* Gom. var., respectively (Table 2). The quantitative carotenoid composition of the algae from these biotopes is compiled in Table 1. Little variation is observed for *O. rubescens* whether cultured [7] or grown in nature. This is also the case comparing results obtained from a previous bloom of *O. agardhii*, green-coloured strain [8], and the recent bloom of *O. agardhii*, red-coloured strain. However, the red-coloured strain of *O. agardhii* had the highest content of oscillaxanthin (**1**) so far reported in blue-green algae [2, 3].

Oscillaxanthin (**1**), identified from its electronic and MS properties [4], was used for stereochemical studies.

In principle, acid hydrolysis (Scheme 1) of oscillaxanthin (**1**) could occur by protonation followed by cleavage according to route (a) providing the chiral aglycone oscillol, or route (b) providing the allylic carbocation. Optically inactive oscillol (**2**) was prepared as a model by DIBAL reduction of the corresponding synthetic 2,2′-dione. Oscillol (**2**) proved very unstable towards acidic conditions resulting in non-polar yellow decomposition products. Consequently acidic hydrolysis of oscillaxanthin (**1**) was abandoned as a route to chiral oscillol.

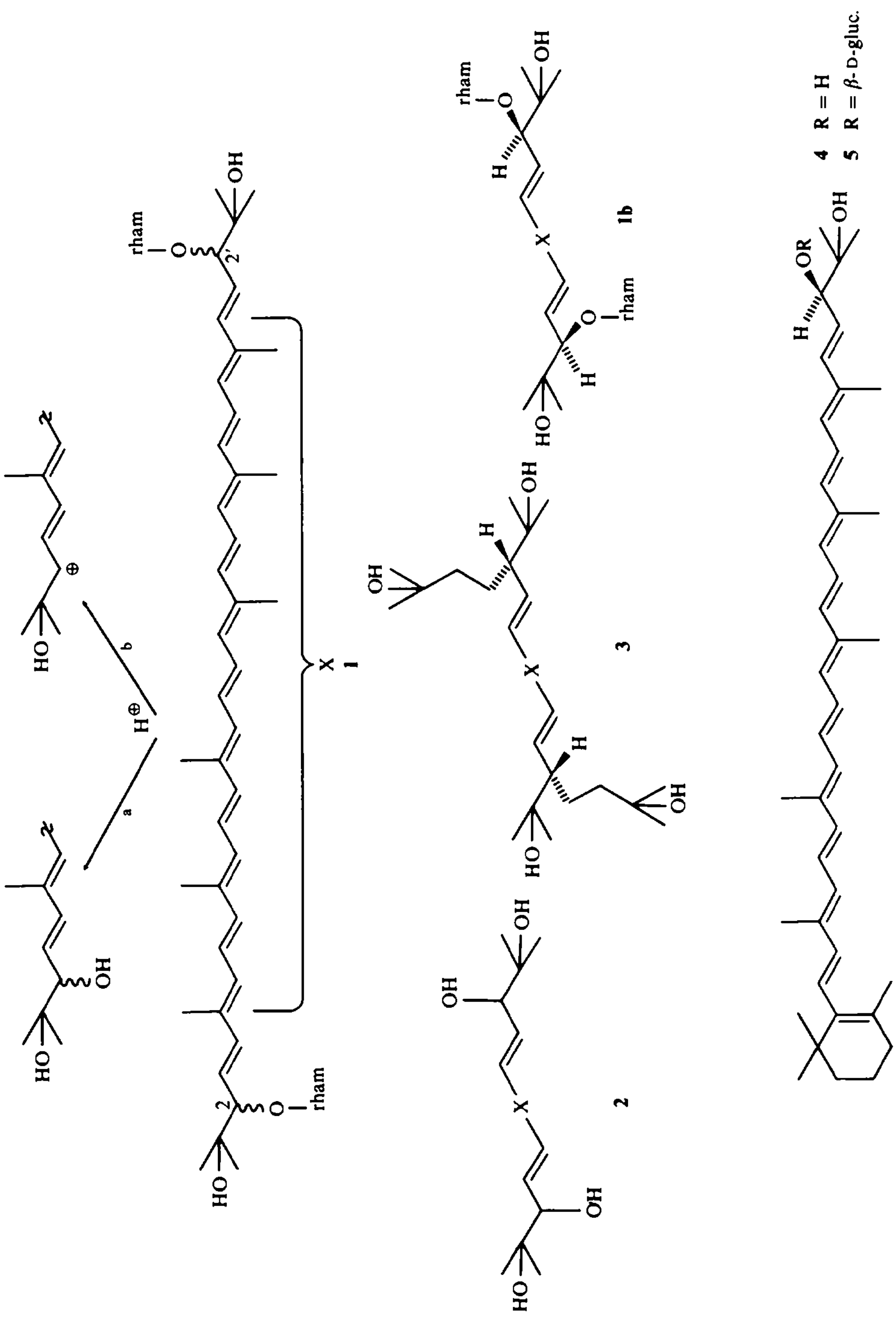
It has been reported [9] that alkaline hydrolysis of tertiary carotenoid glycosides with sodium in ethanol provides intact aglycones. However, oscillaxanthin (**1**) proved to be very stable towards alkaline conditions. If feasible, alkaline hydrolysis probably would have involved inversion at C-2,2′.

Enzymatic hydrolysis of oscillaxanthin (**1**) with α- or β-glucosidase also failed. However, the CD spectrum of oscillaxanthin (**1**) hexaacetate (Fig. 1) provided stereochemical information.

Oscillaxanthin (**1**) hexaacetate exhibited opposite Cotton effect to that of (2*S*,2′*S*)-bacterioruberin (**3**) [10] with the same chromophore. Provided the C_5-substituent at C-2(2′) in bacterioruberin (**3**) was comparable with the sugar residue in oscillaxanthin (**1**) hexaacetate this would indicate opposite configuration at C-2,2′ for oscillaxanthin (**1**).

A better CD model was found in plectaniaxanthin (**4**). Its chirality has recently been assigned as 2′*R* by total synthesis of (2′*S*)-16′,17′-dinorplectaniaxanthin acetonide and CD-correlation with plectaniaxanthin (**4**)

* Part 10 in the series "Carotenoids of Blue-Green Algae". For Part 9 see Hallenstvet *et al.* (1979) *Biochem Syst. Ecol.* **7**, 1.



Scheme 1.

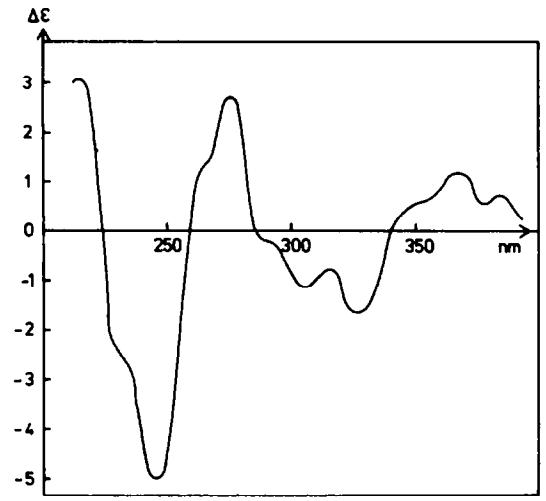


Fig. 1. CD spectrum in EPA solution of oscillaxanthin hexaacetate.

acetonide [11, 12]. Oscillaxanthin (**1**) hexaacetate exhibited the same Cotton effect as (2′*R*)-plectaniaxanthin (**4**) with *ca* 30 nm bathochromic shift, as expected for the shorter chromophore of **4**. Since the second end group of plectaniaxanthin (**4**) is achiral CD-comparison of carotenoids with different chromophore is still justified [13]. It may be argued that comparison of a 2′ol with a 2′-glycoside (as acetate) may not be valid. Natural plectaniaxanthin (**4**) was therefore converted to its β-D-glucoside tetraacetate (**5**) by a Koenigs–Knorr reaction with acetobromoglucose. No change in sign of the Cotton effect for **4** and **5** was observed.

In conclusion CD correlation of oscillaxanthin (**1**) tetraacetate, both with *ent*-bacterioruberin (*ent*-**3**) and with (2′*R*)-plectaniaxanthin-2′-β-D-glucoside (**5**) tetraacetate, supports 2*R*,2′*R*-configuration for oscillaxanthin (**1b**).

EXPERIMENTAL

Biological material. The blue–green algae used for the present study were harvested from natural winter blooms of plankton

Table 1. Carotenoid composition of *O. rubescens* and *O. agardhii*

Carotenoid	*O. rubescens* DC. Clone Staub 51 NIVA/CYA 1 [7]	*O. rubescens* DC.var. Lake Steinsfjord	*O. agardhii* Gom. Lake Aarungen [8]	*O. agardhii* Gom.var Lake Kolbotnvatn
β,β-Carotene	29	37	35	26
β,β-Carotene-3-ol (cryptoxanthin)	4	—	4	—
β,β-Carotene-3,3′-diol (zeaxanthin)	8	9	9	12
β,β-Carotene-4-one (echinenone)	19	19	8	18
3′-Hydroxy-β,β-carotene-4-one	1	trace	0.5	0.6
5,8-Epoxy-5,8-dihydro-β,β-carotene (flavacene≡mutatochrome)	—	—	0.7	—
Myxoxanthophyll	30	20	33	22
Oscillaxanthin (**1**)	10	14	10	23
Carotenoid as % of dry wt	0.28	0.32	0.13	0.36

Table 2. Species examined*

Organism	Locality	Sample	Reference
Oscillatoria agardhii Gom.	Lake Aarungen, Akershus, Norway	Algal bloom, August 1967	18
O. agardhii Gom. var.	Lake Kolbotnvatn, Akershus, Norway	Algal bloom, April 1978	19
O. rubescens DC.	Lake Zürichsee, Switzerland	Algal culture, clone Staub 51, NIVA/CYA 1	20
O. rubescens DC. var.	Lake Steinsfjord, Buskerud, Norway	Algal bloom, April 1978	18

* Unialgal clone cultures of the species investigated are kept in the Culture Collection of Algae at the Norwegian Institute for Water Research.

and stored frozen. The vegetation was dominated by blue-green algae, and microscopical analysis indicated that more than 95% of the plankton vol. in the sample consisted of blue-green algae; the oscillatorians virtually being present as a monoculture. The species discussed in this paper are listed in Table 2.

General methods. These were as commonly employed in the Trondheim laboratory [4, 5, 14].

Pigment isolation. Pigments were extracted with Me_2CO and the crude concentrate chromatographed on a column of acetylated polyamide [15] with C_6H_6 containing increasing amounts of MeOH as eluant, effecting complete separation of myxoxanthophyll and oscillaxanthin. Less polar carotenoids were rechromatographed on Si gel plates. Quantities were determined spectroscopically using $E^{1\%}_{1cm} = 2500$ at λ_{max} in Me_2CO. The relative composition of non-polar carotenoids was also checked by HPLC [14].

Oscillaxanthin (**1**) had λ_{max}467, 496 and 529 nm in Me_2CO and required 15% MeOH in C_6H_6 for elution from acetylated polyamide, total yield *ca* 1.5 mg. Compound **1** was treated with Na in EtOH at 50° [9]. The reaction was monitored by TLC. No new products were isolated after 5 hr. Compound **1** in H_2O containing less than 10% EtOH was treated with (i) α-glucosidase and (ii) β-glucosidase at 30°. No new products were formed during 6 hr.

Oscillaxanthin (**1**) *hexaacetate* was prepared by standard acetylation; *m/e* 1144 (M), M-18, M-106, M-288, 273, 171, 153 [4]; CD Fig. 1.

Oscillol (**2**). Synthetic 1,1′-dihydroxy-1,2,1′,2′-tetrahydro-3,4,3′,4′-tetrahydro-ψ,ψ-carotene-2,2′-dione was reduced with 20% DIBAL in C_6H_6. Compound **2** was isolated by TLC (Si gel); λ_{max}464, 492 and 524 nm in MeOH. Compound **2** was kept in 0.018 N HCl in MeOH for 30 min. Work-up followed by TLC revealed transformation to less polar products. Stronger acid conditions gave rapid decomposition.

(2′R)-Plectaniaxanthin-2′-β-D-glucoside (**5**) *tetraacetate.* (2′*R*)-Plectaniaxanthin (**4**) ex *Plectania coccinea* [16, 11, 12] was submitted to a Koenigs-Knorr reaction with α-D-bromoacetoglucose in Et_2O soln with Ag_2CO_3 as a catalyst and $CaSO_4$ as moisture adsorber [17]. (2′*R*)-Plectaniaxanthin-2′-β-D-glucoside (**5**) tetraacetate was isolated in 10% yield after repeated TLC (Si gel) and had the same electronic spectrum as **4**; *m/e* 898 (M), M − 58, M − 330, 331, 169; CD (EPA soln; Et_2O–*iso*-pentane–EtOH 5:5:2) nm (Δε) 230 (+2), 250 (0), 270 (−1.3), 290 (0), 298 (+0.1) and 330 (−0.7). (2′*R*)-plectaniaxanthin (**4**) had CD nm (Δε) 230 (+1), 245 (0), 270 (−2), 310 (0) and 335 (−0.5).

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