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ABSOLUTE CONFIGURATION OF ALEURIAXANTHIN*

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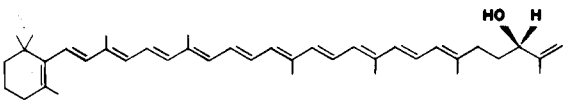
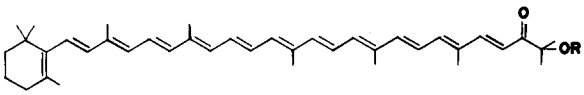
Key Word Index—*Aleuria aurantia*; Ascomycetes; fungus; carotenoid; aleuriaxanthin; absolute configuration; unique carotenoid end group.

Abstract—The chirality of aleuriaxanthin *ex Aleuria aurantia* has been shown to be 2′*R* by means of the modified Horeau method. Other carotenoids with terminal methylene in an acylic end group were not detected in *A. aurantia*.

The constitution of the characteristic pigment of the ascomycete *Aleuria aurantia* has recently been elucidated in our laboratories by means of spectroscopic and chemical methods [1], including total synthesis in racemic form [2]. We now report the chirality at C-2′, solved by means of the modified Horeau method [3,4]. The principle of this method has recently been reviewed in connection with its successful application to configurational assignment of cyclic carotenoids with 2-OH-β-type end groups [5]. Briefly it is based on the partial resolution of a diastereomeric mixture of racemic and meso α-phenylbutyric anhydride by means of an optically active alcohol, which reacts preferentially with one diastereomer. The excess anhydride is reacted with an optically active primary amine, followed by quantitative analysis of the diastereomeric amides by GLC. From the amide formed in excess, the alcohol under examination can be judged to have a particular arrangement of neighbouring groups.

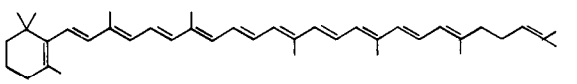


(1)



(2) R = Acyl

(3) R = H



(4)

In the case of aleuriaxanthin (**1**) the *R,S*-amide was formed in excess from (+)-*R*-phenylethylamine and α-phenylbutyric anhydride, as demonstrated by GLC. Under the assumption that the terminal isopropylene group is the most bulky one in the surroundings of the hydroxy group, aleuriaxanthin can be given the *R*-configuration (**1**).

Attempts were made to confirm this assignment by increasing the space requirement of the acyclic end group by hydrogenation. Selective hydrogenation of the terminal methylene was considered difficult, but Horeau analysis of the perhydro compound was also regarded informative. However, under each of the four different hydrogenation conditions used (10% Pd/C in methanol, PtO_2 in ethanol, 5% $Rh–Al_2O_3$ in ethanol [6] or PtO_2–$NaNO_2$–ethanol [7]) considerable hydrogenolysis of aleuriaxanthin (**1**) or its acetate occurred, judged by MS examination of the products. Thus the allylic C—O bond of aleuriaxanthin (**1**) seemed considerably more susceptible to hydrogenation than that of lutein [8].

The hydroxylated end group of aleuriaxanthin (**1**) is so far unique in the carotenoid series. This is also true for terminal methylene in an aliphatic end group. In light of these more recent findings [1,2] the previously reported carotenoid constituents [9,10] of *Aleuria aurantia* were reinvestigated.

Other carotenoids reported in *Aleuria aurantia* are β,β-carotene (26–34% [9], 38% [10]), β,ψ-carotene (**4**, 39–50% [9], 36% [10]), 3,4-didehydro-ψ,ψ-carotene (0–1% [9]), unidentified xanthophylls (1% [9]), an ester of 2′-dehydroplectaniaxanthin (**2**, 2.4% [10]) and free 2′-dehydroplectaniaxanthin (**3**, 0.5% [10]). The identification of free and esterified 2′-dehydroplectaniaxanthin was confirmed by co-chromatography with authentic samples and electronic, IR, ^1H NMR and MS of the free alcohol *cfr.* [11,12]. The large β,ψ-carotene (**4**) fraction was carefully checked. However, no carotene with terminal methylene could be detected either chromatographically, by IR or ^1H NMR of the crystalline β,ψ-carotene (**4**).

Aleuriaxanthin (**1**) represents the first carotenoid with a chiral acyclic end group of established chirality. The Cotton effect of its CD spectrum [1] is very weak in accordance with the long distance and the free rotation of the three single bonds between the polyene chromophore and the chiral center.

Determination of the absolute configuration of other 2′-hydroxy-carotenoids with chiral acyclic end groups such as plectaniaxanthin [11], 2-hydroxy-plectaniaxan-

* No. 10 in the series Fungal Carotenoids. No. 9 (1973) *Acta Chem. Scand.* **27**, 2495.

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thin [13] and the phlei-xanthophylls [14] is being pursued.

EXPERIMENTAL

Materials and methods were as usually employed in the Norwegian laboratory and are summarized elsewhere [15].

Isolation, saponification and acetylation were effected as previously described [1,9], yield *ca* 35 mg cryst. acetylated aleuriaxanthin, *ca* 40 mg cryst. β,ψ-carotene, and *ca* 4 mg free 2'-dehydroplectaniaxanthin (from the ester). Judged by ^{1}H NMR and IR cryst. β,ψ-carotene showed no detectable presence of any carotene with terminal double bond. Evidence for such constituents were neither obtained by chromatography (TLC and circular paper).

Catalytic hydrogenation was carried out with 2–4 mg aliquots of aleuriaxanthin (**1**) and **1**-acetate at room temp. with (a) 10% Pd–C in MeOH, (b) PtO_2 in EtOH, (c) 5% Rh–Al_2O_3 in EtOH [6] and (d) PtO_2–$NaNO_2$ in EtOH [7] until no further hydrogen uptake could be observed (3–48 hr). Product analysis by MS in all cases revealed at least partial hydrogenolysis and complete (a) or partial (b–d) saturation of double bonds.

Horeau experiments. Three Horeau experiments were carried out with aleuriaxanthin (**1**), obtained by alkaline hydrolysis of cryst. aleuriaxanthin acetate directly before use. For each experiment [5] (including parallel reaction with cyclohexanol) three GLC were recorded, from which an average value was calculated. For quantitative GLC packed glass columns 1.5 m × 3 mm, containing 5% OV 17, programmed from 140–250° at 5°/min, N_2-flow 30 ml/min (Expt. 1) and 2 m × 3 mm, 5% OV 225 at 215° isothermal, N_2-flow 20 ml/min (Expts. 2 and 3) were used. The retention times were: *R,R* amide 19.2 min and *R,S* amide 19.8 min on OV 17 and *R,R* amide 15.4 min and *R,S* amide 17.2 min on OV 225. *Results*: 3.2 mg aleuriaxanthin (**1**) in 14 μl dry C_5H_5N and 3.6 μl racemic phenylbutyric anhydride [16] were heated in a sealed Pyrex tube at 40–45° during 75 min. Then 3.6 μl (+) *R*-α-phenylethylamine was added at room temp, the mixture shaken for 15 min and transferred to EtOAc (1–2 ml) for GLC. The parallel reaction was carried out in the same manner but using cyclohexanol (0.6 μl) instead of aleuriaxanthin (**1**). The following ratios were found for the two diastereomeric amides: Expt 1. *R,S*-amide: *R,R*-amide = 1:0,96 (standard reaction with cyclohexanol 1:1.11), corrected ratio by comparison with standard reaction 1:0.96/1.11 = 1:0.86. Expt. 2. corrected value 1:0.87 and Expt. 3. corrected value 1:0.83.

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