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UNUSUAL CARBOHYDRATE PATTERN IN *TRENTEPOHLIA* SPECIES

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Key Word Index—*Cephaleuros virescens*; *Trentepohlia* spp; Chroolepidaceae; polyols; glycerol; erythritol, arabinitol; ribitol; mannitol; volemitol; photosynthesis; accumulation.

Abstract—Four *Trentepohlia* species and the related *Cephaleuros virescens* (Chroolepidaceae, Trentepohliales, Chlorophyceae) photosynthesize and accumulate mannitol, arabinitol, erythritol and glycerol, while *Trentepohlia* spp. additionally synthesize a second pentitol, ribitol (adonitol). *T. umbrina* also contains small amounts of a heptitol, volemitol.

INTRODUCTION

The distribution of polyhydroxy alcohols (polyols, alditols) within the major plant taxa has chemotaxonomic significance [1, 2]. Polyols other than C_5 and C_6 alditols are rarely found as accumulated compounds. Heptitols occur in a few randomly distributed species [3] and tetritols in some species of fungi and algae [2, 4]. Apart from the occurrence of threitol in the flagellate *Ochromonas malhamensis* [5], erythritol is apparently restricted to green algal genera such as *Pleurococcus* and *Trentepohlia* [1, 6], which contribute phycobionts to a wide variety of lichens. *T. aurea* rapidly incorporates photosynthetic carbon into erythritol, ribitol and mannitol [7]. Such simultaneous occurrence of diverse polyols in a single plant species is a remarkable feature. Among the multicellular algae only one species of the genus *Pelvetia* (Phaeophyta) and all species of the genus *Bostrychia* (Rhodophyta) photosynthesize two different alditols [8, 9].

These findings from *T. aurea* and the availability of some other related species provided further opportunity for an experimental assessment of alditol distribution among the representatives of a relatively primitive green algal order, with special regard to alditol accumulation during photosynthetic carbon assimilation.

RESULTS AND DISCUSSION

At least 3 different alditols, erythritol, arabinitol along with mannitol, have been found in all species investigated (Table 1). The *Trentepohlia* spp. also contain ribitol (adonitol), and volemitol was found in *T. umbrina*. In this species the total polyol content was *ca* 4.5% of dry matter. While mannitol (1.7%) represents the major part of all polyols involved, percentages of arabinitol, erythritol, and ribitol (adonitol) are 1.3, 0.9 and 0.5, respectively. Volemitol occurs only in trace amounts.

Table 2 shows the time course in proportional ^{14}C-labelling of the neutral constituents in *T. umbrina* during photosynthetic carbon assimilation from a $H^{14}CO_3^-$ medium. Mannitol as well as arabinitol shows strong ^{14}C-labelling even after a few min exposure to radiocarbon. Kinetics of ^{14}C-labelling, as indicated by percentages of radiocarbon recovered from these compounds, suggest rapid accumulation. Very similar kinetics are found for erythritol and ribitol (adonitol), suggesting that these constituents are also

Table 1. Distribution of polyhydroxy alcohols (alditols) among species of the Chroolepidaceae (Trentepohliaceae)

	Glycerol	Erythritol	Ribitol	Arabinitol	Mannitol	Volemitol
T. aurea	+	+	+	+	+	–
T. umbrina	+	+	+	+	+	+
Trentepohlia sp.*	+	+	+	+	+	–
Trentepohlia sp.†	+	+	+	+	+	–
Cephaleuros virescens	+	+	–	+	+	–

* From Berkeley, CA.
† From Baton Rouge, LA.

Table 2. Time course of ^{14}C-labelling of diverse alditols in *T. umbrina* during photosynthesis in % of total soluble ^{14}C

	Glycerol	Erythritol	Ribitol	Arabinitol	Mannitol	Volemitol
15 min	tr	3.0	3.6	14.0	24.3	tr
30 min	tr	3.2	4.8	19.5	24.7	tr
60 min	tr	12.5	4.1	16.4	42.8	0.6
15 hr	0.9	30.9	3.8	21.2	33.2	3.9

tr = Trace.

accumulated during photosynthetic carbon reduction. In contrast to these observations obtained for the C_4–C_6 alditols, free glycerol as well as volemitol do not appear among the more rapidly ^{14}C-labelled neutral compounds, but show ^{14}C-incorporation after longer periods of photosynthesis (Table 2). The proportional distribution of ^{14}C among the diverse polyhydroxy alcohols of *T. umbrina* is in accordance with the absolute amounts determined from specimens of the same species, at least for shorter incubation periods.

In addition to the polyols, glucose was the only other carbohydrate which incorporated ^{14}C during photosynthesis (5% of total soluble ^{14}C after 30 min, declining thereafter). No sucrose was found in *T. umbrina* or the related species studied.

This pattern of low MW carbohydrates in some *Trentepohlia* spp. and the related *Cephaleuros virescens* is a unique feature as compared with the chemical characters of the majority of other green algae so far investigated. The species investigated here differ from most other Chlorophyta by lacking sucrose as the main constitutive photosynthate. The special characters of the *Trentepohlia* spp. are (i) the occurrence of alditols ranging from C_4 to C_6 or even C_7 along with glycerol, and (ii) the occurrence of two isomeric pentitols, arabinitol and ribitol (adonitol). As far as is known, neither a similar wide range of a tetritol, two pentitols, a hexitol and a heptitol nor the occurrence of two accumulated pentitols have hitherto been reported for any other plant species. Photosynthetic ^{14}C-labelling of erythritol has either been reported for *Trentepohlia*-containing lichens [10]. Arabinitol is for the first time observed as a ^{14}C-labelled photosynthate of an algal species.

Since C_3–C_6 (C_7) polyols were not encountered in *Fritschiella tuberosa* (Chaetophoraceae), the accumulation of alditols may be a useful chemical marker of the Chroolepidaceae (Trentepohliaceae) within the green algal order Chaetophorales (Trentepohliales). Certain structural peculiarities of species of the Chroolepidaceae have recently been discussed [11].

The occurrence of more than one polyhydroxy alcohol (often replacing the constitutive green algal disaccharide sucrose [12]) seems to be a particular feature of aerophilic algae as may be derived from observations with other taxa such as *Pleurococcus* and *Chlorohormidium* [13] or even marine species discussed earlier [8, 9]. However, at present it remains an open question whether the occurrence and biosynthesis of polyols (alditols) is of immediate significance for the autecology of the species involved.

EXPERIMENTAL

Material. *T. aurea* (L.) Martius and *T. umbrina* (Kütz.) Born. were collected in the vicinity of Würzburg and Köln (Germany). *Trentepohlia* spp. and the foliicolous *Cephaleuros virescens* Kunze were kindly supplied by Dr. J. A. West (Berkeley, CA) and Dr. R. L. Chapman (Baton Rouge, LA). *Fritschiella tuberosa* Iyeng. was kindly given by Prof. L. Kies (Hamburg, Germany).

Incubation. Specimens of *T. umbrina* were allowed to photosynthesize ^{14}C from a $H^{14}CO_3^-$ freshwater medium by incubation in a Warburg apparatus under continuous shaking in the light as described earlier [7]. Sufficient CO_2 was present to allow a linear rate of photosynthesis for at least 5 hr. Air-dried specimens stored at −23° for 12 months were fully reactivated after 5–10 hr upon wetting.

Analytical. Soluble, low-MW compounds were extracted from the plant material by repeated treatment with a mixture of $CHCl_3$–MeOH–HCO_2H (6 N) 12:5:3. Neutral compounds were isolated from these extracts by ion exchange chromatography using 10 × 1 cm columns of Dowex 50 and Dowex 1 following a routine procedure. The neutral fraction as well as the crude extracts were further analysed by TLC as described earlier [7, 14]. Polyhydroxy alcohols were separated and identified by repeated cochromatography with authentic substances in all solvents of refs. [14–16]. Quantitative determinations were performed using a periodate oxidation procedure as described in ref. [17] using calibration curves for each polyol.

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