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SYNTHESIS AND BIOLOGICAL ACTIVITY OF (+)-NICOTIANAMINE*

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Key Word Index—*Lycopersicon esculentum*; tomato mutant *chloronerva*; Solanaceae; (2*R*:3′*R*:3″*R*)-*N*-[3-(3-amino-3-carboxypropylamino)-3-carboxypropyl]-azetidine-2-carboxylic acid; (2*R*:3′*R*)-*N*-(3-amino-3-carboxypropyl)-azetidine-2-carboxylic acid; chlorophyll formation.

Abstract—(+)-Nicotianamine and its naturally occurring antipode exhibited the same biological activity with regard to chlorophyll formation of chlorotic leaflets of the mutant *chloronerva*. It is therefore concluded that nicotianamine does not exert its functions by a stereospecific binding to a macromolecular surface.

INTRODUCTION

(−)-Nicotianamine has been identified as the normalizing factor which restores chlorophyll synthesis and growth of the auxotroph mutant *chloronerva* of *Lycopersicon esculentum* Mill. It was suggested that (−)-nicotianamine exerts its function in higher plants as a chelating agent for iron ions [2]. The question is, however, whether this function is only mediated by complex formation within the conductive tissues of the plant or whether (−)-nicotianamine acts as a cofactor of a specific iron carrier, for instance as part of an iron receptor mechanism in the cell membrane, similar to microbial siderophores [3]. If the latter assumption is valid, the steric structure of nicotianamine should play a decisive role in the interactions with the hypothetical macromolecule [4]. Hence (+)-nicotianamine (**1**) which, of course, has the same chelating properties as the naturally occurring antipode, should not reveal biological activity.

RESULTS AND DISCUSSION

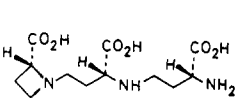
(+)-Nicotianamine (**1**) and the structurally related (2*R*:3′*R*) - *N* - 3 - amino - 3 - carboxypropyl) - azetidine - 2 - carboxylic acid (**2**) have been obtained from *R*-azetidine-2-carboxylic acid in 2 and 7% yields, respectively, by a similar procedure described for the antipodes [5]. In contrast to (−)-nicotianamine [5], **1** was obtained pure by a modified isolation procedure.

(+)-Nicotianamine (**1**) and (−)-nicotianamine exhibited the same biological activity with respect to chlorophyll formation of chlorotic leaflets of the mutant *chloronerva* down to a concentration of 1.7×10^{-5} M, the lowest concentration tested. The minimum amount of substance that gave a positive response was about 10^{-8} mol/plant. It is therefore concluded that nicotianamine does not exert its function via a stereospecific binding to a macromolecular surface such as a membrane or a receptor protein. It is rather more likely, that nicotianamine performs its action within the tissue as an iron chelating agent by transporting iron to the sites of chlorophyll synthesis and plastid development in the intercostal areas of the leaf.

As in the case of its antipode, (2*R*:3′*R*) - *N* - (3 - amino - 3 - carboxypropyl) - azetidine - 2 - carboxylic acid (**2**) proved to be biologically inactive.

EXPERIMENTAL

(2*R*:3′*R*:3″*R*) - *N* - [3 - (3 - *Amino* - 3 - *carboxypropylamino*) - 3 - *carboxypropyl*] - *azetidine* - 2 - *carboxylic acid* [(+)-*nicotianamine* (**1**)]. A soln of 998 mg *R*-azetidine-2-carboxylic acid [6] in 50 ml 0.1 M NaOH was refluxed for 24 hr under N_2. The cooled soln passed a column of 50 g Dowex 50 WX 4, 100–200 mesh, analytical grade. Elution of amino acids was carried out with 1 M NH_3. The eluate was chromatographed using a column of Sephadex G-10 (1.15 × 177 cm) with 0.05 M NH_3 as eluant (3 ml fractions). Here and in subsequent separations **1** was detected by TLC [2]. The residue of fractions 28–33 (407 mg) was subjected to prep.



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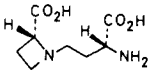

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*Part 12 in the series "The 'Normalizing Factor' for the Tomato Mutant *chloronerva*". For part 11, see ref. [1].

PC [*n*-BuOH–H_2O–HOAc (12:5:3), Schleicher & Schüll paper 2315, 1.8 mg material/cm, descending paper strip chromatography, 2 days; **1** migrated *ca* 20 cm; detection with ninhydrin] and ion exchange chromatography (Dowex 50 WX 8, 200–400 mesh, analytical grade, 0.1 M pyridine formate buffer pH 3.3, column 2.2 × 36 cm, 20-ml fractions). Fractions 15–24 gave 20 mg **1** from H_2O–EtOH with decomp. above 250° and $[\alpha]_D^{22} + 43.8°$ (H_2O, *c* 0.51). The IR spectrum (KBr) proved to be identical with that of (−)-nicotianamine.

(2R:3'R) - N - (3 - *Amino* - 3 - *carboxypropyl*) - *azetidine* - 2 - *carboxylic acid* (**2**). The residue of the Sephadex G-10 fractions 34–37 (306 mg, see above) was purified by ion exchange chromatography (conditions as above). Fractions 10–20 gave 68 mg needles from H_2O–EtOH with decomp. above 240° and $[\alpha]_D^{22} + 76.4°$ (H_2O, *c* 0.81). The IR spectrum (KBr) proved to be identical with that published for its antipode [5].

Biological test. Seedlings of *Lycopersicon esculentum* Mill. cv 'Bonner Beste' mutant *chloronerva* were raised in quartz sand and transferred to a nutrient soln after the first leaf became visible. Composition of the nutrient soln was: $Ca(NO_3)_2$ 5×10^{-3}; KNO_3 5×10^{-3}; KH_2PO_4 1×10^{-3}; $MgSO_4$ 1×10^{-3}; H_3BO_3 4.6×10^{-5}; FeEDTA 5×10^{-6} M. Plants were cultivated in a growth cabinet at a photoperiod of 16 hr light/8 hr dark; temp. 25/20°; r.h. 70 ± 5%; light intensity (photosynthetically active radiation 400–700 nm) 300–310 $\mu E/m^2$ per sec; lamp type: fluorescent tubes, 90% 'warm white', 10% 'Lumoflor' (VEB Narva, Berlin). The biological test was performed after dissolution of the respective substance in 0.05% Tween 20 (Atlas-Goldschmidt GmbH, Essen, West Germany) by painting the chlorotic leaflets 5× per day with a smooth brush. Each treatment was performed with five seedlings. The response was considered positive when a change of the chlorotic leaflets to a normal green colour was observed which happened at the lowest concn. used within 4 days.

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3-[(7Z)-HEXADECENYL]-4-METHYLFURAN-2,5-DIONE FROM *PIPTOPORUS AUSTRALIENSIS*

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Key Word Index—*Piptoporus australiensis*; Polyporaceae; basidiomycete; structural determination; citraconic anhydride derivative; 3-[(7*Z*)-hexadecenyl]-4-methylfuran-2,5-dione.

Abstract—The structure of a new citraconic anhydride derivative from *Piptoporus australiensis* is established by spectroscopic and chemical methods as 3-[(7*Z*)-hexadecenyl]-4-methylfuran-2,5-dione.

INTRODUCTION

Recently we reported [1] the isolation of several novel polyolefinic compounds which are responsible for pigmentation in the bright orange fruiting body of the basidiomycete *Piptoporus australiensis* (Wakefield) Cunningham. We describe here the isolation from the same fungus of a colourless metabolite to which we assign the substituted citraconic anhydride structure (**1**). This is the first reported occurrence of a citraconic anhydride derivative in a basidiomycete. Only two other organisms are known to produce monomeric* substituted citraconic anhy-

*The 'nonadrides', a group of compounds isolated from various *fungi imperfecti* [4] are derived by dimerization of short side-chain derivatives of citraconic anhydride [5].