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NICOTINE INHIBITION OF CAROTENOID CYCLIZATION IN *CUCURBITA FICIFOLIA* COTYLEDONS

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Key Word Index—*Cucurbita ficifolia*, Cucurbitaceae, gourd; nicotine, carotenoids, lycopene cyclization, chlorophyll synthesis

Abstract—Nicotine inhibits carotenoid cyclization in greening cucurbit cotyledons resulting in the accumulation of acyclic and monocyclic carotenes Chlorophyll synthesis is also inhibited by the alkaloid

INTRODUCTION

NICOTINE was initially reported to inhibit the cyclization of the acyclic carotenoid lycopene into bicyclic carotenes in two species of *Mycobacterium*. γ-carotene with a single cyclohexylidene ring also accumulated in the presence of nicotine suggesting that nicotine inhibited at least two cyclization reactions. The effect was reversible and the normal bicyclic carotenes formed when the nicotine was washed from the cells.[1] Recently the cyclization of lycopene by cells washed free of nicotine and incubated under anerobic conditions was reported.[2] The effectiveness of nicotine as a carotenoid cyclization inhibitor has been confirmed with a species of *Flavobacterium*,[3] with *Myxococcus fulvus*[4] and with the fungus *Phycomyces blakesleeanus*.[3] The hydrochloride of nicotine, however, did not cause lycopene accumulation in grapefruit or orange and it was suggested that nicotine is not active in a broad spectrum of plants.[5] In order to determine the effectiveness of nicotine as a carotenoid inhibitor in a group of higher plants, experiments were carried out with greening cucurbit cotyledons.

RESULTS AND DISCUSSION

A minimum of 7 mM nicotine is required to cause accumulation of lycopene and monocyclic carotenes in greening cotyledons of gourd (*Cucurbita ficifolia* Bouché). The alkaloid is also effective in other species of *Cucurbita* including pumpkin, Zucchini, summer and winter squashes and in *Cucumis sativus* L. (cucumber). In gourd, β-carotene is normally the major carotene formed in greening cotyledons. In the presence of nicotine lycopene, γ-carotene and δ-carotene also form (Table 1). The major monocyclic carotene formed in gourd is δ-carotene which has an α-cyclohexylidene ring.

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[2] BATRA, P P, GLEASON, JR, R M and LOUDA, S W (1973) *Phytochemistry* **12**, 1309
[3] GOODWIN, T W (1971) *Biochem J.* **123**, 293
[4] KLEINIG, H and REICHENBACH, H (1973) *Biochim Biophys Acta* **306**, 249
[5] YOKOYAMA, H, DEBENEDICT, C, COGGINS, C W and HENNING, G L (1972) *Phytochemistry* **11**, 1721

The minimum concentration of nicotine required for inhibition of carotenoid cyclization in *Cucurbita* species is much greater than that required in bacteria where concentrations as low as 0·1 mM[1] and 0·02 mM[4] have been used with some effect. For maximum lycopene accumulation in bacteria, however, about 5 mM nicotine is required.[1,4]

Since nicotine has previously been used only in nonphotosynthetic organisms and tissue, its inhibitory effect on chlorophyll synthesis is also of interest (Table 1) Concentrations of 7·5 mM nicotine and below are inhibitory to chlorophyll synthesis, but not to total carotenoid synthesis suggesting that the inhibitory action of the alkaloid is in the porphyrin biosynthetic pathway or after geranyl-geranyl pyrophosphate in phytol synthesis This is indicated because both carotenoids and phytol are synthesized by the same route up to geranylgeranyl pyrophosphate

TABLE 1 THE EFFECT OF NICOTINE ON THE FORMATION OF PIGMENTS IN GREENING GOURD COTYLEDONS EXPOSED TO 24 HR OF LIGHT

		μg/g fresh wt		
Pigment	Cyclohexylidene rings	H_2O	Nicotine 7·5 mM	Nicotine 15 mM
β-Carotene	2	9·7	2·4	1·9
γ- and δ-Carotenes	1	–	4·9	4·8
Lycopene	0	–	8·3	9·8
Xanthophylls		36·5	31·0	19·2
Total carotenoid		46·2	46·6	35·7
Chlorophyll		354	238	151

A 10 mM solution of nicotine has a pH of 8·7. It is therefore of interest that the action of nicotine varies with pH. In an experiment in which 10 mM solutions of nicotine were buffered in a range from pH 4 to 11 it was found that the alkaloid had no effect on carotenoid cyclization and little effect on chlorophyll synthesis under acid conditions The effectiveness of nicotine as a carotenoid cyclization inhibitor and a chlorophyll synthesis inhibitor increased from neutral to more alkaline conditions The pronounced effect of pH on nicotine action may be due to structural considerations of the molecule, to effects on membrane permeability or both The lack of effectiveness of nicotine as a carotenoid cyclization inhibitor in citrus fruits[5] may be due to the acidity of these fruits

EXPERIMENTAL

Cucurbit seeds were germinated in the dark in moist vermiculite for 6–7 days The cotyledons were removed and placed in Petri dishes with 10 ml of distilled H_2O or with nicotine solutions Buffered solutions contained 30 mM Tris and 30 mM sodium, potassium phosphate The amount of carotenoid present before light exposure was typically 10 mg/g fresh weight about evenly divided between β-carotene and xanthophylls Petri dishes were incubated for 24 hr under a fluorescent lamp at 2000 lx, the cotyledons blotted with paper towels and the fresh wt determined Samples were frozen and the pigments extracted at a later time

Pigments were extracted by grinding in a homogenizer with acetone Chlorophyll was determined by the method of Vernon[6] Extracts were saponified, the carotenoids extracted in petrol, and the pigments separated on a column of activated alumina[7] The amount of each polyene eluted from the column was calculated from its extinction coefficient For xanthophylls an extinction coefficient of 2500 at 450 nm was assumed[8] δ-Carotene

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[7] BRITTON, G and GOODWIN, T W (1971) *Methods of Enzymology* (MCCORMICK, D B and WRIGHT L D, eds), Vol XVIII pt C, pp 654–701, Academic Press, New York

[8] GOODWIN, T W (1955) *Modern Methods of Plant Analysis* (PAECH, K and TRACEY M V, eds) Vol III, pp 272–311, Springer, Berlin

was identified by its position on the column, elution characteristics and spectral properties (λ_{max} in petrol 430, 455 and 488 nm)

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