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SYNTHETIC REGULATORS OF CAROTENOID BIOSYNTHESIS IN *CITRUS PARADISI**

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Key Word Index—*Citrus paradisi*; Rutaceae; Marsh seedless grapefruit; carotenoid biosynthesis; bioregulators; partition coefficient; Hammett constant; tertiary amine; lycopene.

Abstract—The ability of 16 amines to induce carotenoid biosynthesis in Marsh seedless grapefruit is correlated with the octanol–water partition coefficient and the Hammett constants. The compounds fall into three series: p-$RC_6H_4COOCH_2CH_2NEt_2$ (R = H, NH_2, CN, NO_2, MeO, Me, *tert*-Bu, F, Cl, Br), p-$RC_6H_4CH_2NEt_2$ (R = H, Me, NO_2), and $RC_6H_4OCH_2CH_2NEt_2$ (R = *o*-Me, *m*-Me, *p*-Me). Total carotene content increased up to 12-fold. Lycopene, not normally accumulated, became a major pigment. The benzoates caused up to a 24-fold increase in the β-carotene content. Except for the larger accumulation of cyclic carotenes, the mode of action of these amines appears to be similar to that of 2-(4-chlorophenylthio)triethylamine hydrochloride.

INTRODUCTION

Tertiary amines of the general formula RCH_2NEt_2 markedly affect carotenogenesis in citrus [1–6]. These bioregulators cause a large increase in the total carotene content with lycopene (ψ,ψ-carotene) becoming a major pigment. This effect is believed to be caused by the derepression of a gene regulating the synthesis of a specific enzyme(s) and the inhibition of the cyclase(s) in the biosynthetic pathway of the carotenoids. The previously determined [5] relation between the ability to stimulate carotenoid biosynthesis and the logarithm of the octanol–water partition coefficient (log *P*) is confirmed by this report. The compounds studied fall into three series: p-$RC_6H_4COOCH_2CH_2NEt_2$, R = NH_2 (**1**), CN (**2**), H (**3**), NO_2 (**4**), MeO (**5**), F (**6**), Me (**7**), Cl (**8**), Br (**9**) and *tert*-Bu (**10**); p-$RC_6H_4CH_2NEt_2$, R = H (**11**), Me (**12**) and NO_2 (**13**); and $RC_6H_4OCH_2CH_2NEt_2$, R = *o*-Me (**14**), *m*-Me (**15**) and *p*-Me (**16**). The mode of action of these amines is similar to previously studied amines except that several of the benzoates cause a substantial increase in the cyclic as well as in the acyclic carotenes.

RESULTS AND DISCUSSION

Control fruit retained the normal light yellow colour. Treated fruit with the greatest lycopene accumulation became an intense red colour. Because the test compounds did not penetrate into the interior of the fruit, this method of treatment caused colour enhancement only in the flavedo, while the endocarp remained uncoloured. The peel remained healthy on all fruit except those treated with **10** which developed a brown scarring of part of the peel.

Table 1 gives the results of treatment with **1–10**. The response caused by **1–10** is similar to that previously observed for other amines [3–6] except for the much larger accumulation of the cyclic carotenes, γ-, α- and particularly β-carotene (β,ψ-, β,ε- and β,β-carotene). Treatment with **11–16** (Tables 2 and 3) did not increase the cyclic carotenes. Treatment with **4, 8** and **9** did show neurosporene (7,8-dihydro-ψ,ψ-carotene) but it was unresolved from the lycopene. There was no lycopene in any of the control fruits or the fruit treated with **10**. The lycopene found after treatment with **14** was only tentatively identified and might be due to slight traces of **15** and **16**. Treatment with **11** and **16** gave responses similar to those previously observed [5]. Compound **5** caused a definite increase in the cyclic carotenes which was not seen before [4]. The cyclase was probably inhibited more by the higher concentration (~0.4 M) used in the previous test.

Tables 1 and 2 also give the values of log P and the Hammett constants for the substituent group in the *meta* position (σ_m) [7]. The log P was calculated as previously [5] starting with the value of ethyl benzoate [8] for **1–10** and by use of the π values of the substituents for the benzoic acids [9] except for **10** which was calculated by adding methyl groups to **7** and **1** [10]. Log P for **12** and **13** were calculated from the value for **11** [5] and the substituent value for the benzyl alcohols [9]. Compounds **1–10** show a generally increasing effectiveness with log P except for **2, 4** and **10**. The bulky *tert*-Bu group in conjuction with the carboxy group probably prevents **10** from binding at the active site(s) and accounts for the complete lack of any induced lycopene accumulation. The failure to cause a response may also be due in part to the peel damage observed with higher log P values [5]. The greater than expected effectiveness

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Table 1. Effect of **1–10** at 0.1 M on the carotene content of the flavedo of Marsh seedless grapefruit (μg/g dry wt)

	Control	Treatment 1	2	3	4	5	6	7	8	9	10
Phytofluene	173	191	192	229	245	199	244	234	228	233	204
ζ-Carotene	38.6	44.7	60.7	57.6	64.5	44.9	62.5	62.5	60.4	61.1	51.0
Neurosporene	1.52	0.62	0.95	1.23		1.05	1.43	2.01			1.97
Lycopene		1.14	83.6	17.5	215	170	245	285	474	507	
γ-Carotene	0.38	0.37	3.67	1.48	3.82	1.94	3.04	3.80	3.18	2.91	1.14
α-Carotene	0.53	0.53	0.84	1.76	3.86	2.62	2.82	5.54	4.01	4.22	0.40
β-Carotene	1.31	1.51	27.4	12.0	25.7	6.64	13.6	25.0	17.3	32.4	2.05
Total carotenes	215	240	369	320	557	426	572	618	787	841	261
Total xanthophylls	33.7	35.9	39.8	38.7	45.0	44.6	48.5	42.8	45.3	44.6	46.5
Log P		1.54	2.75	3.06	3.08	3.14	3.25	3.48	3.93	4.04	4.58
σ_m		−0.16	0.56	0.00	0.71	0.12	0.34	−0.07	0.37	0.39	−0.10

of **2** and **4** is probably due to the increased interaction with the active site(s) caused by the greater electron withdrawing effect of the substituents. This effect is also observed with **11**, **12** and **13**. Compound **13** is much more effective than **12**. The electron withdrawing effect of the nitro group is more effective at increasing the inducing ability of the compound than the greater increase in log P caused by the methyl group. σ_m is used instead of σ_p because it is probably the *ortho* position of the aromatic ring that is involved with the binding at the active site(s). This is the case with the phenoxyacetic acids [11]. Also Table 3 shows that substitution of a methyl group at the *ortho* position almost completely eliminates the inducing ability of the compound. Substitution at the *meta* position also causes a reduction in the inducing ability. With the methyl group in the *para* position the compound is a very powerful inducer. The same effect is observed with the strongly electron withdrawing chloro group, as compared to the electron releasing methyl group, for *p*-, *m*- and *o*-chlorophenoxytriethylamine [6].

These results agree with those previously observed. The carotenogenesis inducing bioregulators have the general formula RCH_2NEt_2. The inducing ability increases with log P up to about 4.5 at which point the compounds begin to cause tissue damage. Those inducers with an aromatic ring show increased activity if an electron withdrawing substituent is placed at the *para* position. These compounds all give results similar to those caused by previously reported inducers with lycopene as the main carotene induced, but in addition the benzoates also cause a much greater accumulation of β-carotene than previously seen.

Table 2. Effect of **11**, **12** and **13** at 0.2 M on the carotene content of the flavedo of Marsh seedless grapefruit (μg/g dry wt)

	Control	Treatment 11	12	13
Phytofluene	36.0	38.1	39.5	54.7
ζ-Carotene	5.00	6.96	7.73	23.3
Neurosporene	0.28	0.93	1.12	1.87
Lycopene		4.93	22.1	234
γ-Carotene		0.31	0.40	1.71
α-Carotene	0.48	0.47	0.43	0.69
β-Carotene	1.96	1.90	1.35	0.60
Total carotenes	43.7	53.6	72.6	317
Total xanthophylls	22.2	21.6	22.7	26.3
Log P		3.07	3.55	3.23
σ_m		0.00	−0.07	0.71

EXPERIMENTAL

Post-harvest treatment of fruit. Each sample consisted of 6 Marsh seedless grapefruit harvested at the fully mature stage. Fruits used to test **1–10** were harvested at a different time and place than those used to test **11–16**. Compounds **1–10** and **11–13** were applied as 0.1 and 0.2 M solns, respectively, of the free amines in *iso*-PrOH, while **14–16** were used as 0.1 M solns of the hydrochlorides in *iso*-PrOH. The control was treated with *iso*-PrOH. The soln was poured over the surface of the fruit to give complete coverage. The fruit were allowed to drain and then moved to a clean surface to air dry for several hr before being placed in polyethylene bags and stored at room temp. (~21°) for 2 weeks.

Isolation and identification of the pigments. Pigments were isolated and identified by published methods [5]. A portion of ground flavedo was dried *in vacuo* at 65° to give the dry wt.

Compounds **1–10.** To a soln of 0.22 mol of *N,N*-diethylethanolamine in 100 ml $CHCl_3$ stirred in an ice-bath, was added dropwise 0.2 mol of the acyl chloride corresponding to the desired ester. Solid acyl chlorides were dissolved in half the $CHCl_3$ and added dropwise. *p*-Cyanobenzoyl chloride was added as a solid because of its low solubility in $CHCl_3$. After addition, the soln was removed from the ice-bath and allowed to stand 24 hr at room temp. An additional 100 ml $CHCl_3$ was added and the soln was washed ×5 with 250 ml satd $NaHCO_3$ and then ×2 with 250 ml H_2O. The soln was dried

Table 3. Effect of **14**, **15** and **16** at 0.1 M on the carotene content of the flavedo of Marsh seedless grapefruit (μg/g dry wt)

	Control	14	15	16
Phytofluene	40.2	39.3	36.1	52.7
ζ-Carotene	4.66	3.26	4.00	17.7
Neurosporene	0.26	0.79	1.14	1.77
Lycopene		0.43	24.4	516
γ-Carotene		0.22	0.60	0.83
α-Carotene	0.52	0.34	0.61	0.28
β-Carotene	1.25	0.86	0.59	0.48
Total carotenes	46.9	45.2	67.4	589
Total xanthophylls	21.8	21.2	24.3	25.1

over K_2CO_3 and the $CHCl_3$ removed with a rotary evaporator. The ester was used without further purification.

Compounds **12**, **13**, **15** *and* **16**. Published methods [5] were used to synthesize **12** and **13** from the corresponding bromides (the reaction is vigorous and requires initial cooling in an ice-bath) and **15** and **16** from the corresponding phenols.

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