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SYNTHETIC BIOREGULATORS OF POLY-*CIS* CAROTENOID BIOSYNTHESIS*

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Key Word Index—*Citrus paradisi*; Rutaceae; Marsh white seedless grapefruit; carotenoid biosynthesis; bioregulators; secondary amines; poly-*cis* carotenoids; carotenoids; prolycopene.

Abstract—Seventeen new bioregulators were synthesized and tested for their ability to induce the biosynthesis of poly-*cis* carotenes in the flavedo of Marsh white seedless grapefruit. The effects of these new bioregulators are the same as that of the previously reported dibenzylamines, but several of the new compounds are more effective and cause the accumulation of up to 162 μg/g dry wt of poly-*cis* carotenes in the flavedo as compared to the maximum of 74 μg/g dry wt observed previously. The compounds tested were substituted *N*-benzyl furfurylamines, *N*-benzyl, *N*-methyl furfurylamines and *N*-alkyl, *N*-methyl benzylamines. They demonstrate the ability of tertiary as well as secondary amines to stimulate the formation of poly-*cis* carotenes. The interaction of *N*-(4-bromobenzyl) furfurylamine, one of the more effective of the new compounds, with lycopene and β-carotene inducers is also reported.

INTRODUCTION

Previously, the ability of substituted dibenzylamines to induce the biosynthesis of poly-*cis* carotenes has been reported [1]. Since dibenzylamines were not as effective at inducing pigment formation as the lycopene (ψ, ψ-carotene) [2, 3] and β-carotene (β, β-carotene) [4] inducers, 17 new bioregulators were synthesized and tested on Marsh white seedless grapefruit in order to find more effective inducers and to study the structural characteristics of compounds causing poly-*cis* carotene induction. The compounds tested were: *N*-R furfurylamine, R = benzyl (**1**), 4-methylbenzyl (**2**), 4-chlorobenzyl (**3**), 4-bromobenzyl (**4**), 4-nitrobenzyl (**5**), phenethyl (**6**), 2-phenoxyethyl (**7**) and 2-(4-bromophenoxy)ethyl (**8**); *N*-methyl, *N*-R furfurylamine, R = benzyl (**9**), 4-bromobenzyl (**10**) and phenethyl (**11**); *N*-methyl, *N*-R benzylamine, R = *n*-butyl (**12**), *n*-pentyl (**13**), *n*-hexyl (**14**), *n*-heptyl (**15**) and *n*-octyl (**16**); and *N*-(*n*-hexyl) benzylamine (**17**). The interaction of **4** with the lycopene inducer, 2-(phenoxy)triethylamine (**18**) [2], and β-carotene inducer, 2-diethylaminoethyl hexanoate (**19**) [4], is also discussed.

RESULTS AND DISCUSSION

Treated grapefruit become a bright orange colour rather than the red colour caused by the lycopene inducers because of the hypsochromic shift in going from the all-*trans* carotenes to the poly-*cis* carotenes. Carotenogenesis was only stimulated in the flavedo; the endocarp was unaffected.

The results obtained with those bioregulators are shown in Tables 1–3. Prolycopene was identified by its spectrum and chromatographic behaviour but was not compared to prolycopene from Tangerine tomato fruit whose absolute stereochemistry has been established [5]. Our procedure would not separate ζ-carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene) from the poly-*cis* isomer, so the increase in ζ-carotene could be due to the formation of poly-*cis*-ζ-carotene.

The pattern of accumulation of the poly-*cis* carotenes is the same as that caused by the dibenzylamines [1]. The *N*-benzyl furfurylamines are more effective than the corresponding dibenzylamines. The only anomaly is compound **8** which acted as an inducer of both poly-*cis* carotenes and lycopene. It also inhibited the cyclase(s), a characteristic of lycopene inducers [2, 3], and prevented the formation of poly-*cis*-γ-carotene I and II. The effectiveness of **9-11** shows that *N*-methylation of the corresponding secondary amines (**1**, **4** and **6**) does not eliminate activity but tends to enhance activity. This enhancement of activity is especially evident with the *N*-alkyl, *N*-methyl benzylamines. While **17** is only weakly active, **14** is a very good inducer.

Table 4 shows the results of applying **4** simultaneously with, or 2 days after, the fruit is treated with **18**, a lycopene inducer, and **19**, a β-carotene inducer. Simultaneous treatment with **4** and **18** or **4** and **19** caused a marked decrease in the amount of carotenes that would normally be produced by **18** or **19** alone. The poly-*cis* carotenes induced by **4** are also reduced but not to such a high degree. When **4** was applied 2 days after the initial treatment with **18**, considerable lycopene was formed because of the initial rapid accumulation [4] but less than treatment with **18** alone because of the antagonistic effect of **4** and **18** against each other. The fruit treated initially with **19** and then after 2 days with **4** accumulated

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Table 1. Effect of compounds **1–8** at 0.1 M on the carotene content of the flavedo of Marsh white seedless grapefruit (μg/g dry wt)

	Control	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Phytofluene	19.78	22.03	21.17	25.03	31.03	19.44	25.63	31.70	28.32
α-Carotene	0.34	0.24	0.33	0.23	0.20	0.21	0.22	0.30	0.29
β-Carotene	1.51	1.26	1.36	1.31	1.46	0.93	1.48	1.20	0.95
ζ-Carotene	2.58	11.72	6.18	18.71	27.61	2.26	22.79	12.57	14.94
Poly-*cis*-γ-carotene I		0.95	0.45	2.24	2.72		2.01	1.25	
Proneurosporene		8.41	3.42	15.17	21.42	0.44	18.30	10.56	5.07
Prolycopene		11.40	5.23	32.88	46.53	0.72	41.47	20.42	4.99
γ-Carotene									0.36
cis-Lycopenes		7.31	3.81	14.84	23.37	1.13	17.52	8.70	3.78
Neurosporene	1.17					0.72			2.64
Poly-*cis*-γ-carotene II		4.82	1.68	10.49	14.45		8.46	5.45	
Unknown 453		2.03	1.16	2.05	3.70		3.54	2.49	
Lycopene									32.39
Total carotenes	25.38	70.17	44.79	122.95	172.49	25.85	141.42	94.64	93.73
Total xanthopylls	19.13	22.18	22.40	24.78	30.85	20.62	27.09	24.73	22.14

Table 2. Effect of compounds **1**, **4**, **6** and **9–11** at 0.1 M on the carotene content of the flavedo of Marsh white seedless grapefruit (μg/g dry wt)

	Control	**1**	**9**	**4**	**10**	**6**	**11**
Phytofluene	22.13	28.83	31.54	35.63	33.30	30.94	37.05
α-Carotene	0.13	0.19	0.20	0.23	0.30	0.14	0.17
β-Carotene	0.57	1.11	1.46	1.06	1.28	1.50	1.73
ζ-Carotene	2.68	20.65	29.76	21.07	21.51	36.95	35.80
Poly-*cis*-γ-carotene I		2.82	4.05	4.47	3.08	3.01	5.55
Proneurosporene		14.65	21.36	19.34	14.51	29.98	25.48
Prolycopene		27.59	48.15	45.04	33.30	79.52	65.32
cis-Lycopenes		18.06	29.04	24.81	26.21	30.99	39.18
Poly-*cis*-γ-carotene II		13.56	21.03	21.74	10.08	18.09	21.39
Unknown 453		7.12	8.55	5.67	8.10	5.92	9.09
Total carotenes	25.51	134.58	195.14	179.05	151.67	231.18	240.76
Total xanthopylls	26.33	35.71	41.95	36.71	38.87	32.10	46.75

almost as much β-carotene as fruit treated with **19** alone. This is because **19** normally causes a rapid increase in the lycopene content the first 2 days and then becomes inactive allowing the lycopene to be converted into β-carotene [4]. This also shows that **4** does not inhibit the cyclization of the accumulated lycopene to β-carotene. This, along with the fact that **4** stimulates the production of the cyclic poly-*cis*-γ-carotenes I and II, shows that the poly-*cis* inducers are not inhibitors of the cyclase(s) as is the case with the lycopene inducers [2, 3].

EXPERIMENTAL

Fruit samples. Marsh white seedless grapefruit were harvested in January for the tests shown in Tables 1 and 4 and April for Tables 2 and 3. All samples consisted of six fruit.

Post-harvest treatment of fruit. Solns of all test compounds were prepared at 0.1 M in *iso*-PrOH except **19** which was 0.2 M. **1–11** and **17–19** were applied as the free amines, while **12–16** were used as the hydrochlorides. The treatment is as previously described [1]. The flavedo was removed **14** days after the initial treatment.

Isolation, identification and quantification of the pigment. Determined by absorption spectra, iodine-catalysed photoisomerization and chromatographic behaviour [1]. Compounds **1**, **2** and **5–8** were synthesized by the same method as **4** [6] from the bromides and furfurylamine. Compound **3** was similarly synthesized from the chloride. Compounds **9**, **10** and **11** were synthesized from **1**, **4** and **6**, respectively by methylation with formic acid and formaldehyde [7]. Compounds **12–16** were synthesized like *N*-(*n*-decyl), *N*-methyl benzylamine [6]. Compound **17** was synthesized by the same method as the dibenzylamines [1] from hexylbromide and benzylamine. Compounds **18** and **19** were synthesized as previously [2, 4]. The purity and identities of the amine hydrochlorides were tested using HPLC and mass spec-

Table 3. Effect of compounds **12–17** at 0.1 M on the carotene content of the flavedo of Marsh white seedless grapefruit (μg/g dry wt)

	Control	**12**	**13**	**14**	**15**	**16**	**17**
Phytofluene	24.86	29.40	24.03	35.20	28.07	34.12	13.55
α-Carotene	0.50	0.36	0.23	0.23	0.21	0.31	0.14
β-Carotene	0.95	1.01	1.70	1.64	0.77	0.93	1.10
ζ-Carotene	2.73	3.85	7.26	21.24	20.86	24.99	4.77
Poly-*cis*-γ-carotene I			0.86	2.94	2.90	3.15	1.19
Proneurosporene		1.77	5.01	16.08	16.38	18.80	3.68
Prolycopene		2.66	9.45	32.57	36.06	26.44	8.96
cis-Lycopenes		3.63	4.75	10.94	13.26	12.33	4.64
Poly-*cis*-γ-carotene II		0.39	3.58	11.17	8.03	9.83	4.20
Unknown 453		0.20	1.21	2.09	3.58	2.03	0.82
Total carotenes	29.04	43.27	58.08	134.10	130.12	132.93	43.05
Total xanthophylls	25.83	25.04	27.90	33.85	27.89	27.18	19.85

Table 4. Effect of the interaction of 0.1 M **4** with 0.1 M **18** or 0.2 M **19** on the carotene content of the flavedo of Marsh white seedless grapefruit (μg/g dry wt)

	Control	**18**	**19**	**4**	**4 + 18**	**4 + 19**	**4 + 18***	**4 + 19***	**4***
Phytofluene	26.61	31.99	19.37	39.32	31.93	30.63	32.10	27.35	37.95
α-Carotene	0.18	0.11	4.07	0.16	0.31	0.39	0.32	2.31	0.14
β-Carotene	0.77	0.69	68.07	1.18	0.87	4.66	0.86	50.00	1.38
ζ-Carotene	2.79	13.30	10.31	30.01	18.77	15.49	13.24	10.51	32.88
Poly-*cis*-γ-carotene I				3.85		1.81		2.79	4.53
Proneurosporene				23.91	13.47	8.47	5.86	4.21	26.58
Prolycopene				49.13	38.93	24.77	22.46	15.20	67.20
γ-Carotene		2.77	4.26						
cis-Lycopenes				28.08	23.44	20.02	18.73	20.47	22.82
Neurosporene			1.21						
Poly-*cis*-γ-carotene				24.55		6.67			23.35
Unknown 453				5.62		3.81			6.87
Lycopene		266.12	15.99		24.68	trace	102.29	15.03	
Total carotenes	30.35	314.98	123.28	205.81	152.40	116.72	196.86	147.87	223.70
Total xanthophylls	20.02	25.61	31.87	43.65	24.73	33.74	31.20	38.54	40.90

*The fruit was initially treated with **18**, **19** or *iso*-PrOH and then after 2 days with **4**.

trometry. HPLC was performed using a 250 × 4.6 mm, Supelco 5 μm reverse phase C_{18} column. The solvent was 45% MeOH in aq. 0.25 N H_3PO_4 adjusted to pH 3 with triethylamine. Absorbance was monitored at 206 nm. Retention times relative to **1** are: **2** (1.52), **3** (1.63), **4** (1.97), **5** (0.90), **6** (1.31), **7** (1.34), **8** (3.28), **9** (1.04), **10** (2.24), **11** (1.51), **12** (1.17), **13** (1.86), **14** (3.68), **15** (7.67), **16** (16.89) and **17** (3.34). Mass spectra were determined on a VG Micromass 7070F using the solid insertion probe. The hydrochlorides disassociate in the source to give the spectra of the free amines. EI (70 eV) gave the following results: **1** *m/z* 187 (M^+, 24%), **2** 201 (18), **3** 221 (22), **4** 265 (15), **5** 232 (33), **6** 201 (1), **7** 217 (7), **8** 295 (4), **9** 201 (44), **10** 279 (9), **11** 215 (1), **12** 177 (6), **13** 191 (6), **14** 205 (5), **15** 219 (4), **16** 233 (4) and **17** 191 (4).

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REFERENCES

1. Poling, S. M., Hsu, W.-J. and Yokayama, H. (1980) *Phytochemistry* **19**, 1677.
2. Poling, S. M., Hsu, W.-J. and Yokoyama, H. (1975) *Phytochemistry* **14**, 1933.
3. Poling, S. M., Hsu, W.-J. and Yokoyama, H. (1976) *Phytochemistry* **15**, 1685.

4. Poling, S. M., Hsu, W.-J., Koehrn, F. J. and Yokoyama H. (1977) *Phytochemistry* **16**, 551.
5. Clough, J. M. and Pattenden, G. (1979) *J. Chem. Soc. Chem. Commun.* 616.
6. Echols, L. C., Maier, V. P., Poling, S. M. and Sterling, P. R. (1981) *Phytochemistry* **20**, 433.
7. Boissier, J. R., Batouis, R. and Dumont C. (1966) *Ann. Pharm. Fr.* **24**, 57.