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Changes in Some Carotenoids and Apocarotenoids during Flower Development in *Boronia megastigma* (Nees)

CHRIS M. COOPER,*,† NOEL W. DAVIES,‡ AND ROBERT C. MENARY†

School of Agricultural Science and Central Science Laboratory, University of Tasmania, G.P.O. Box 252-54, Hobart, Tasmania, Australia

The carotenoid profile of an acetone extract from the flowers and leaves of *Boronia megastigma* (Nees) was examined. A comparison was made of the major carotenoids found in boronia flowers and leaves. The C-40 carotenoids β-carotene, zeaxanthin, lutein, and neoxanthin were positively identified in boronia flowers using known standards, UV−vis spectra, and mass spectrometry. Two other carotenoids were tentatively assigned as the palmitic acid ester of 3-hydroxy-10′-apocaroten-10′-oic acid and 9,15,9′-tri-*cis*-ζ-carotene. Additionally, changes in the levels of C-40 carotenoids, C-27 apocarotenoids, and β-ionone during flower development were measured. Significant increases in β-carotene and apocarotenoids that could be derived from cleavage in the 9,10-position, including β-ionone and various C-27 apocarotenoids, were observed at the time of flower opening. An increase in lutein, which is derived through an alternative biosynthetic pathway, was not observed during flower opening, thus indicating the possibility that the β-carotene pathway was activated during flower opening in boronia. The understanding of these processes may assist in optimizing harvest and postharvest processes useful to the boronia extraction industry.

KEYWORDS: ***Boronia megastigma*** **(Nees); carotenoids; C-27 apocarotenoids; C-13 norisoprenoids; 9,15,9′-tri-*cis*-ζ-carotene; 3-palmitoyloxy-10′-apo-β-caroten-10′-oic acid**

INTRODUCTION

An extract from the flowers of *Boronia megastigma* (Nees) was first obtained during the early part of the 20th century from wild plants (*1, 2*). An extract from this species is currently extracted from commercial clones as part of the Tasmanian essential oils industry. The absolute is used worldwide as a natural flavor enhancer in foods containing berry fruit products. The extract has a wide range of high-value aroma compounds including β-ionone as one of the main components (*3*−*5*), and an ongoing research program, conducted in our laboratory, has led to advances in relation to overall yield and component content in the flower extract. This included substantial progress during the 1990s that was based on the work of MacTavish (*6*) in developing protocols for harvesting and postharvest processes that gave increases in the yield of volatiles.

The experimental program was strategically focused on β-ionone production due to the exceptional flavor and aroma value of this compound (*7*) and its low flavor and aroma threshold (*8*). MacTavish and Menary (*9*) found that both the extract as a percentage of fresh flower weight and the concentration of β-ionone increased until 70% of flowers were open. This finding led to detailed recommendations for field harvesting of the different commercial clones (*10*) to maximize the β-ionone content of the extract. In addition, harvested flowers incubated at between 12 and 25 °C for up to 24 h showed increased levels of extract of up to 25% (*11*) with increases in some compounds including β-ionone. These laboratory experiments led to the development of a pilot-scale process with an on-farm application (*12*).

Our interest in the carotenoid content of *B. megastigma* (Nees) was derived from speculation concerning the role of these compounds as metabolic precursors of β-ionone and other C-13 norisoprenoids which may have importance in enhancing the overall aroma/flavor profile of the extract. The role of a site-specific 9,10-carotenoid cleavage dioxygenase in the biosynthesis of β-ionone and other C-13 norisoprenoids has been proposed since the 1980s without any direct evidence (*13*). More recently, site-specific cleavage in the 9,10-position has been demonstrated in several plants including star fruit (*14*), nectarines (*15*), and grape berries (*16*). The 9,10-specific cleavage enzymes are part of a family of carotenoid cleavage enzymes with significant structural homology and diverse site and substrate specificities (*17, 18*).

β-Carotene is a possible precursor of β-ionone, and this compound has been detected in boronia flowers (*6*). Additionally, evidence for site-specific cleavage of carotenoids in boronia

* Author to whom correspondence should be addressed (telephone ++61 3 62 262724; fax ++ 61 3 62 267609; e-mail christopher.cooper@utas.edu.au).

† School of Agricultural Science.

‡ Central Science Laboratory.

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was obtained through the detection of five C-27 apocarotenoids in boronia flowers (*19*). These compounds along with a range of C-13 norisoprenoids previously characterized in boronia (*3*−*5*) structurally match sections of known C-40 carotenoids. Their presence in flowers provided strong evidence that β-ionone was derived from a C-40 carotenoid precursor through site-specific cleavage. Additionally, the presence of hydroxylated C-27 apocarotenoids in the flowers allowed speculation that 9,10-specific cleavage of xanthophylls also occurred in boronia. Therefore, we intended to undertake a more thorough investigation to identify boronia carotenoids and measure the changes of these compounds and their cleavage products during flower development. A further understanding of the biochemistry of boronia carotenoids may contribute to an increased yield of a range of C-13 norisoprenoids in the extract through both harvest and postharvest technologies.

MATERIALS AND METHODS

Materials. Flowers from *B. megastigma* (Nees) clones 3 and 250 developed by the University of Tasmania and grown in southern Tasmania were used. β-Ionone (97.2% by GC) and β-carotene standards were obtained from Sigma. HPLC grade solvents were used for all extraction and chromatographic purposes. All other reagents were of analytical grade.

Sampling Technique. A representative harvest sample (approximately 1 kg) from each of two clones was supplied to the laboratory. Buds and flowers from the whole sample were separated with a sieve and then further selected with forceps to eliminate any overlap between the stages. Three separate samples were taken from each stage. The procedure resulted in 12 samples, 3 replicates of each of the 4 stages.

Flower Development Stages. Four different stages of development (**Table 1**) were selected to maximize observations at the time of bud opening. Selection of these stages allowed a more detailed examination of changes than was undertaken by MacTavish (*6*) during flower opening by including the newly opened bud stage. The large bud stage just prior to opening (stage 2) in this study was equivalent to the large bud stage (stage 4) in the studies performed by MacTavish (*6*).

Plant Extraction. A small-scale extraction method based on the method described by Cooper et al. (*19*) was used for the extraction of carotenoids and β-ionone. Typically, flowers or buds (200−300 mg) were homogenized using an Ultraturrex (T25 basic, Ika labortechnik, setting 6), fitted with a 10 mm head, with 2.5 mL of acetone in the presence of 125 mg of calcium carbonate. Following centrifugation at 10000*g* (Beckman J2-21 M/E, rotor 20.1) the pellet was extracted twice more with 2.5 mL of acetone, and the supernatants were combined. The combined acetone fractions were diluted to a total volume of 9 mL with acetone prior to analysis by HPLC-MS. All operations were at 4 °C under pale yellow or reduced light.

HPLC. A Waters Alliance 2690 HPLC fitted with a Waters Nova-Pak 150 × 3.9 mm i.d. C18 column and an Alltech Econosphere C18 guard column was used to achieve chromatographic separation of β-ionone, C-40 carotenoids, and C-27 apocarotenoids. Two different programs were used for analytical purposes. Separation of clone 3 flowers was achieved with program B, whereas separation of clone 250 flowers was achieved with program A.

Program A. The solvents used to achieve separation were (A) acetonitrile, (B) water, (C) methanol, and (D) hexane. Initial conditions were 50% A/50% B/0% C/0% D, which were held for 2 min and followed by a linear gradient to 85% A/0% B/15% C/0% D at 15 min. A further linear gradient to 0% A/0% B/85% C/15% D at 25 min was then held for 10 min. The column was re-equilibrated to start conditions over 19 min using a linear gradient to 0% A/0% B/100% C/0% D at 35.1 min and a further linear gradient to 50% A/50% B/0% C/0% D at 38 min, which was held for a further 7 min. The flow rate was 1 mL/min.

Table 1. Description of Flower Development Stages Analyzed in This Study

flower stage	description
1	small buds approximately 3−5 mm in diameter
2	large buds approximately 5−7 mm in diameter
3	newly opened flowers that were 1−3 mm open
4	flowers that were >3 mm open

Program B. The solvents used to achieve separation were (A) acetonitrile, (B) water, (C) methanol, and (D) hexane. Starting conditions were 50% A/50% B/0% C/0% D. This proporation was held for 2 min and followed by a linear gradient to 85% A/0% B/15% C/0% D at 22 min. A further program to 0% A/0% B/85% C/15% D at 32 min was held for 8 min. Re-equilibration was through 0% A/0% B/100% C/0% D at 40.1 min and 50% A/50% B/0% C/0% D at 43 min, which was held for a further 6 min. The flow rate was 1 mL/min.

HPLC-MS. Positive ion mass spectral data were obtained with a Finnigan LCQ equipped with an atmospheric pressure chemical ionization (APCI) ion source. Settings were as follows: sheath gas, 60 psi; auxiliary gas, 15 psi; vaporizer temperature, 450 °C; discharge current, 6 μA; capillary temperature, 170 °C; capillary voltage, 20 V. Scanning usually occurred over the *m*/*z* range of 100−1200. However, the signals for the apocarotenoids reported here were maximized by shortening the *m*/*z* range to 100−680. Data-dependent MS/MS scans were also collected from the most intense ion in each spectrum with a default collision energy of 25%.

Data Analysis. *HPLC.* UV−vis chromatograms at 292 nm (β-ionone), 430 nm (apocarotenoids), and 451 nm (β-carotene) were extracted from the data acquired using Waters Millenium software. Data were recorded from 250−700 nm every 1 s at 1.2 nm resolution. β-Carotene and β-ionone were measured quantitatively with known standards, whereas lutein was measured by relating the β-carotene and lutein extinction coefficients. The other carotenoids and apocarotenoid levels are expressed as relative to β-carotene through using the extinction coefficient for β-carotene in calculations. That data are therefore a measure of relative changes for each compound during flower development but do not allow absolute analyte levels to be compared directly.

Statistical Analysis. Analysis of variance was used to test for differences between the four flower development stages.

RESULTS AND DISCUSSION

Identification of Boronia Carotenoids. Following a previous finding that C-27 apocarotenoids were present in the flowers of *B. megastigma* (*19*), a more extensive assessment of boronia carotenoids was undertaken. The main carotenoids in an acetone extract obtained from both leaves and mature boronia flowers were separated using HPLC. The chromatographic peaks were investigated through comparison of known retention times, examination of their UV−vis spectra, and mass spectroscopy. An examination of the carotenoid profile of leaves revealed a typical leaf carotenoid profile (*20*) with neoxanthin, violaxanthin, lutein, and β-carotene present as the main carotenoids, similarly in the leaves from two different boronia clones (3 and 250). Fully developed boronia flowers, however, had a much more complex carotenoid profile, and **Figure 1** shows a chromatogram at 430 nm of the profile in the flowers of clone 250 boronia. In addition to the five C-27 apocarotenoids, previously identified in boronia flowers, the C-40 carotenoids β-carotene, zeaxanthin, lutein, and neoxanthin were identified in the flowers.

The profile presented in **Figure 1** additionally indicated the presence of several unidentified carotenoids. Although many of the chromatographic peaks overlapped, a further four carotenoid peaks were sufficiently resolved (**Figure 1**, peaks 4, 5, 13, and 15) to allow examination of the UV−vis spectra (**Figure 2**) and MS data. Peaks 4, 5, and 15 gave UV−vis spectra that indicated they had similar chromophores. The position of the main absorbance maxima (II and III) at

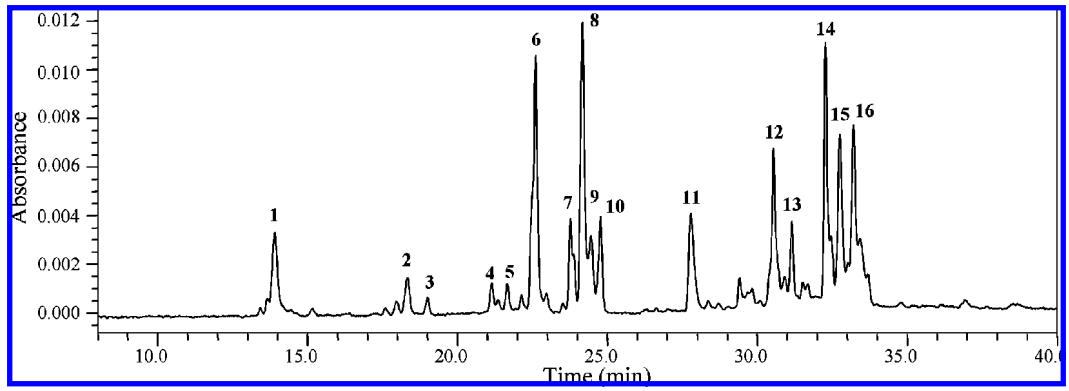

Figure 1. HPLC chromatogram at 430 nm of a carotenoid extract of open boronia flowers. The compounds were assigned the following identities: 1, hydroxy-10′-apocaroten-10′-oic acid; 2, neoxanthin (mixed peak); 3, methyl hydroxy-10′-apocaroten-10′-oate; 4, unknown carotenoid; 5, unknown carotenoid; 6, 10′-apocaroten-10′-oic acid; 7, 10′-apocaroten-10′-al; 8, lutein; 9, zeaxanthin; 10, methyl 10′-apocaroten-10′-oate; 11, chlorophyll *b*; 12, chlorophyll degradation product; 13, 3-palmitoyloxy-10′-apocaroten-10′-oic acid; 14, chlorophyll degradation product; 15, 9,15,9′-tri-*cis*-ζ-carotene; 16, *all-trans*-β,β-carotene.

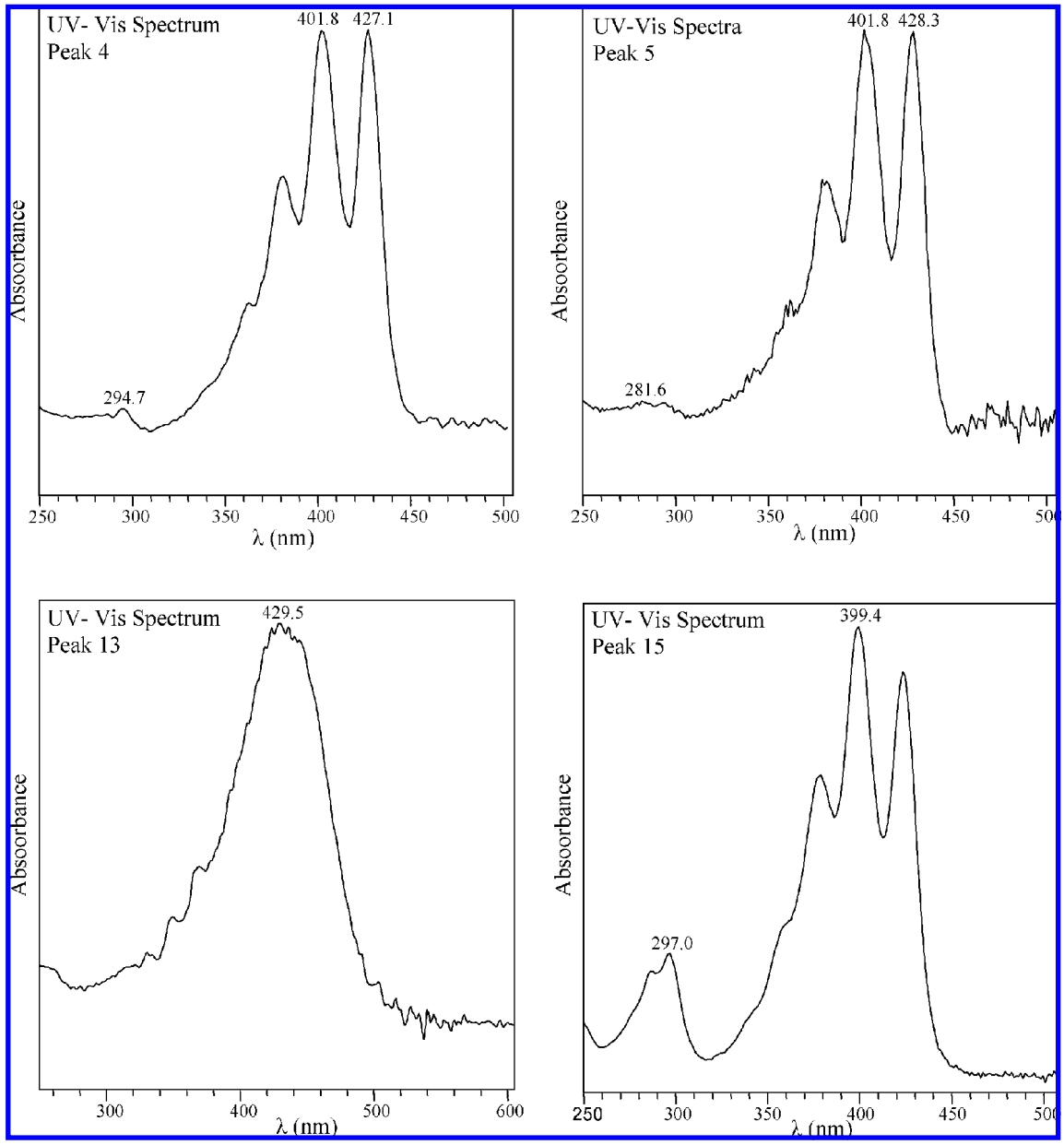

Figure 2. UV−vis spectra of carotenoid peaks 4, 5, 13, and 15 in the HPLC chromatogram (**Figure 1**) of a carotenoid extract from boronia.

approximately 399−402 and 423−428 nm was consistent with a chromophore containing seven conjugated double bonds (*21*). In addition, mass spectral data indicated that the carotenoids which produced peaks 4 and 5 both had a MW of 600, but there

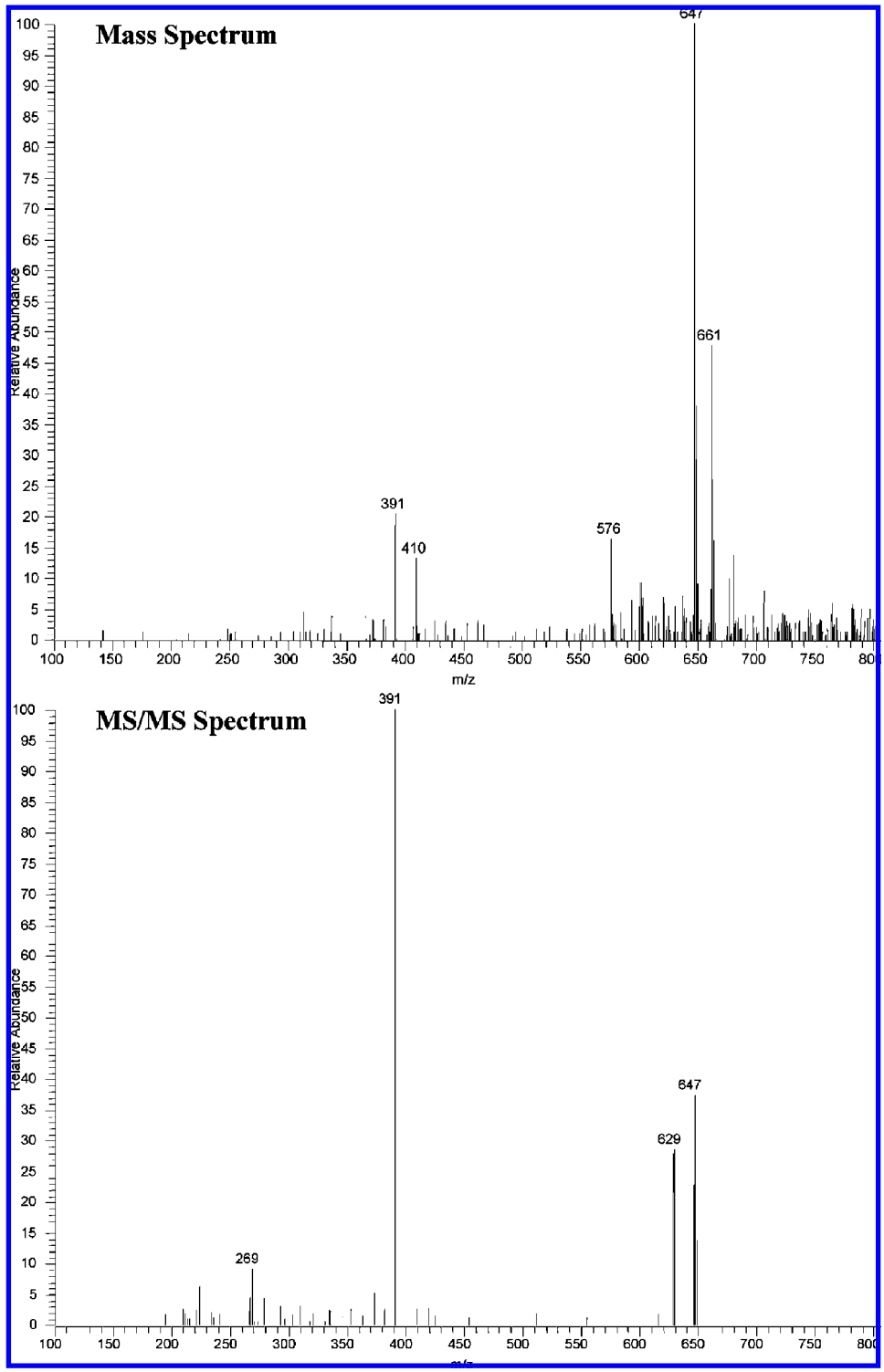

Figure 3. MS and MS^2 spectra for the proposed 3-palmitoyloxy-10′-apocaroten-10′-oic acid (peak 13).

were insufficient data available for unequivocal identification of these compounds.

The elution position (in the hydrocarbon region) and UV−vis spectrum of peak 15 were consistent with its being a *cis*-ζ-carotene (*22*−*24*). The UV−vis spectrum, including the presence of a high double-cis peak (%A_B/A_{II} = 26%) with maxima at 285 and 297 nm and the %III/II ratio of 75%, was consistent with UV−vis data supplied by both Breitenback and Sandmann (*22*) and Li et al. (*23*) for 9,15,9′-tri-*cis*-ζ-carotene. This contrasted with UV−vis data for 9,9′-di-*cis*-ζ-carotene, which is produced through light isomerization of 9,15,9′-tri-*cis*-ζ-carotene as an intermediate step in the biosynthesis of lycopene from phytoene (*22*). In addition, the APCI MS data confirmed the ζ-carotene molecular weight of 540.

The UV−vis spectrum for peak 13 (**Figure 2**) showed a broad single absorption maximum at 430 nm consistent with its being a C-27 apocarotenoid (*19*). The same compound was also detected in boronia absolute, and a mass spectrometric analysis (**Figure 3**) indicated the compound had a molecular weight of 646. This was consistent with a tentative assignment of the compound as 3-palmitoyloxy-10′-apocaroten-10′-oic acid. In corroboration, the MS^2 spectrum gave an intense product ion at *m*/*z* 391 (consistent with dehydrated 3-hydroxy-10′-apo-β-caroten-10′-oic acid) resulting from the loss of palmitic acid, a

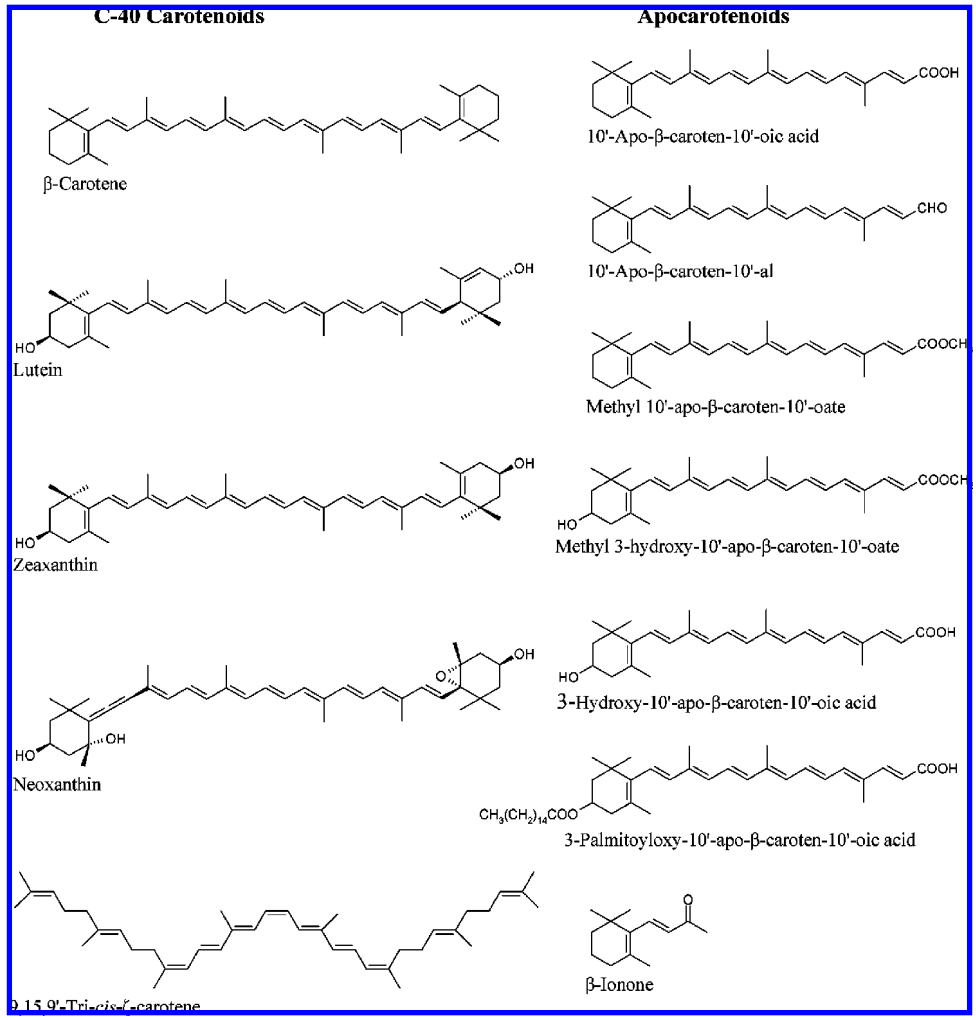

Figure 4. Structures of detected and proposed carotenoids and apocarotenoids in boronia.

typical fragmentation for carotenoid esters (*25*). Esters of palmitic acid are commonly found in plants, and the *Carotenoid Handbook* (*26*) lists zeaxanthin, lutein, and taraxanthin esters of palmitic acid. Palmitic acid was also one of the main fatty acids bound to xanthophylls in potatoes (*27*), to β-cryptoxanthin in various fruits and vegetables (*25*), and to various xanthophylls in fruit extracts (*28*). Additionally, it has been demonstrated that carotenoid esters of fatty acids elute at a later time on C-18 columns than the parent carotenoid (*25*, *28*), and this elution pattern was observed in boronia, further supporting the proposed assignment. This may be the first example of a palmitic acid esters of an apocarotenoid occurring in plants.

Changes during Flower Development. When possible, given the complexity of the chromatograms, the changes in C-40 carotenoids, C-27 apocarotenoids, β-ionone, and some unknown carotenoids were measured quantitatively during flower development in both clones 3 and 250. The structures of these compounds are represented in **Figure 4**, and the data are presented in **Table 2**. Measurement of β-carotene levels during flower development showed that this compound was present in small buds (stage 1). Small changes were then apparent with a marked increase occurring between the development stages associated with just opened buds (stage 3) and the open flowers (stage 4). The increase at this stage was especially apparent in clone 3 flowers. The changes for β-carotene contrasted with the situation that was apparent for lutein which showed a significant increase during bud development (between stages 1 and 2) with a constant level maintained during the later stages of flower development.

The concentration of β-carotene on a wet weight basis in open flowers (stage 4) ranged from 42 mg/kg in clone 3 to 12 mg/kg in clone 250. The levels in clone 3 were equivalent to the levels observed in *Gentiana lutea* (*29*), in which total carotenoids were 766 mg/kg (dry weight basis) in fully open flowers. In that flower β-carotene levels were 25%, whereas lutein levels were 35% of the total. The lutein level in open boronia flowers (stage 4) was 53 mg/kg in clone 3 and 47 mg/kg in clone 250 flowers. The biosynthesis of β-carotene and lutein occur via a branch in the pathway of carotenoid biosyntesis (*29*) and the data presented here supported the possibility of a metabolic shift toward β-carotene biosynthesis and away from lutein production in boronia. This has been observed during flower development in *G. lutea* (*29*). That study additionally demonstrated an up-regulation at the transcription level of lycopene β-cyclase, which leads to β-carotene and a concomitant decrease in the transcript levels of lycopene ε-cyclase, which leads to the biosynthesis of lutein via α-carotene. In contrast, marigold flowers (*30*) produced large amounts of lutein during flower development. Increases in carotenoid levels or an up-regulation of genes associated with carotenoid biosynthesis during flower development has been observed in a range of other plant species including chrysanthemum (*31*) and tomato (*32*).

Changes in the relative levels of the C-27 apocarotenoids and other carotenoids were based on β-carotene equivalence. The

Table 2. Changes in the Level of Carotenoids and Cleavage Products during Flower Development[a]

	clone	stage 1	stage 2	stage 3	stage 4
C-40 carotenoids					
β-carotene	3	7.0a ± 0.5	9.0a ± 0.5	14.1a ± 5.0	41.9b ± 5.6
	250	2.7a ± 0.6	6.0b ± 0.4	6.8b ± 0.2	12.4c ± 1.3
lutein	3	43.5a ± 1.0	59.5b ± 0.9	53.4b ± 3.9	53.4b ± 1.0
	250	32.6a ± 0.6	48.9b ± 5.2	53.6b ± 2.5	47.0b ± 0.9
cis-ζ-carotene*	3	not detected	1.8a ± 0.04	9.1a ± 5.1	31.5b ± 3.8
	250	not detected	not detected	not detected	17.8 ± 0.5
unknown carotenoids					
peak 4*	3	no data	no data	no data	no data
	250	4.8a ± 0.01	3.5b ± 0.3	3.0c ± 0.1	1.8d ± 0.04
peak 5*	3	no data	no data	no data	no data
	250	5.1a ± 0.1	3.8b ± 0.3	3.2c ± 0.2	1.8d ± 0.1
apocarotenoids					
10′-apocaroten-10′-oic acid*	3	not detected	5.0a ± 0.5	9.8b ± 1.4	28.0c ± 0.7
	250	not detected	4.8a ± 0.5	11.0b ± 0.1	22.0c ± 0.6
10′-apocaroten-10′-al*	3	not detected	3.6a ± 0.2	5.7b ± 0.4	9.6c ± 0.3
	250	not detected	1.9a ± 0.2	3.9b ± 0.2	8.0c ± 0.3
methyl 10′-apocaroten-10′-oate*	3	not detected	not detected	not detected	8.1 ± 0.6
	250	not detected	1.1a ± 0.1	2.1b ± 0.1	6.5c ± 0.2
hydroxy 10′-apocaroten-10′-oic acid*	3	not detected	trace	2.3a ± 0.5	14.6b ± 0.3
	250	not detected	0.5a ± 0.1	1.2b ± 0.2	8.0c ± 0.3
3-palmitoyloxy-10′-apocaroten-10′-oic acid*	3	no data	no data	no data	no data
	250	not detected	1.8a ± 0.2	3.2b ± 0.4	4.6c ± 0.1
β-ionone	3	not detected	178.3a ± 14.6	420.1b ± 13.8	175.5a ± 12.9
	250	not detected	190.6a ± 27.9	507.9b ± 1.9	254.9a ± 17.2

[a] The mean of triplicates and standard error are shown. The data are expressed as mg/kg of fresh flowers, which is an equivalence based on the extinction coefficient of β-carotene. Means showing a common letter are not significantly different at $\alpha = 0.05$.

results showed similar patterns of increase with no apocarotenoids present in the small bud stage. 10′-Apocaroten-10′-oic acid increased significantly between the large bud (stage 2) and newly opening buds (stage 3) in both clones. Further significant increases for open flowers (stage 4) were observed with clone 3 flowers showing a 3-fold increase. Levels of 10′-apocaroten-10′-al and methyl 10′-apocaroten-10′-oate also increased significantly during flower development, with the highest levels occurring in open flowers in both clones. The presence of the boronia methyl esters of C-27 apocarotenoic acids is not reported elsewhere. However, methylation of carboxyl groups has been commonly observed in plants (*33*). The data for changes in hydroxy 10′-apocaroten-10′-oic acid levels during flower development showed that small amounts were present in large buds with higher levels at the newly opening bud stage (stage 3). The largest increase occurred between newly opening buds (stage 3) and open flowers (stage 4) in both clones.

Increases were also observed during flower development for the putative ζ-carotene and palmitoyl ester. The appearance of 3-palmitoyloxy-10′-apocaroten-10′-oic acid correlated with the first appearance of the positively identified C-27 apocarotenoids, supporting the rationale that this compound is a C-27 apocarotenoid ester. In clone 3 flowers, ζ-carotene first appeared in large buds (stage 2), increasing to 31 mg/kg (β-carotene equivalent) in open flowers (stage 4). A different pattern was observed in clone 250 flowers, with this compound present only at the open flower stage. *cis*-ζ-Carotene is an intermediate in C-40 carotenoid biosynthesis, and the observation matched the larger increases in β-carotene observed for clone 3 flowers. Increases in *cis*-ζ-carotene alongside accumulation of C-13 norisoprenoids were also observed in the arbuscular mycorrhizal roots of *Zea mays* and *Medicago truncatula* by Fester et al. (*34*).

Carotenoid Cleavage in Boronia. There is considerable evidence for enzyme-mediated site-specific cleavage of boronia carotenoids in the 9,10-position. The strongest evidence for this is that the known carotenoid cleavage products found in boronia, C-27 apocarotenoids and C-13 norisoprenoids (*3*−*5*), are indicative of this site-specific cleavage. In particular, β-ionone is structurally matched with β-carotene. Additionally, the ionols and ionones in boronia that are oxygenated in the 3-position on the ionone ring (e.g., 3-hydroxyionone) are readily matched in structural terms with lutein and zeaxanthin, which have now been detected in boronia flowers (see **Figure 1**). Certainly the increase in hydroxy 10′-apocaroten-10′-oic acid during flower development supported the rationale that carotenoid cleavage of xanthophylls was occurring in boronia. The degradation pathways proposed for carotenoids in boronia have been similarly discussed in other studies including saffron, gardenia fruits, and apple leaves (*35*); watermelons and tomato (*36*); and rose flowers (*37*). A chemically based mechanism for cleavage would presumably result in a different pattern of cleavage products with the appearance of apocarotenoids with different chain lengths through its nonspecificity.

However, it is interesting that, despite extensive research into the identification of boronia volatiles, a C-14 compound corresponding to cleavage of a C-40 carotenoid in both the 9,10- and 9′,10′-positions has not been detected. Equivalent compounds have been found in other plants including rosafluene in roses (*38*) and mycorradicin in mycorrhizal roots (*34*). Since 2002 considerable evidence for a carotenoid cleavage dioxygenase (CCD) with specific activity for cleavage in the 9,10-position, as part of a family of cleavage ennymes in plants with different specificities, has been accumulated (*17*, *18*). Recently, Floss et al. (*39*), working with mycorrhizal roots of *M. truncatula*, presented evidence that carotenoid cleavage in plants may occur via two consecutive steps whereby CCD1 catalyzes the cleavage of C-27 apocarotenoids in cytosol following the initial cleavage of a C-40 carotenoid in plastids. Certainly there is no evidence in boronia, based on metabolite accumulation for dual cleavage of C-40 carotenoids. Elucidation of the carotenoid cleavage process in boronia may have a significant role to play in understanding the role of specific carotenoid cleavage enzymes in plants.

Consideration of β-Ionone Changes. β-Ionone levels reached a maximum in opening flowers (stage 3) with the levels reducing to less than half of that in the open flowers (stage 4) for both

clones. This contrasted with the large increases in β-carotene in open flowers. A possible reason for this is that β-ionone release from flowers begins soon after flower opening (stage 3). This further reinforced the work of MacTavish and Menary (*10*) that led to a recommendation that harvest occur before all of the flowers on each plant are mature. The rate of biosynthesis of plant volatiles versus the rate of release from the plant is a complex process. Release is dependent on several factors including the volatility of the particular compound and compartmentalization factors relating to the site of biosynthesis and the mechanism of excretion (*33*). Other factors relating to biosynthesis of volatiles include the down-regulation of volatile biosynthesis following pollination, which has been demonstrated in petunia and snapdragon flowers (*40*). This is unlikely to be a factor in relation to the Tasmanian boronia harvest as the pollination vector (*3*) is not found in Tasmania.

The increase in β-ionone, and indeed the C-27 apocarotenoids and C-40 carotenoid precursors, as part of an activation of carotenoid biosynthesis and concomitant cleavage of C-40 carotenoids at the time of flower opening, was consistent with a number of experimental observations made by MacTavish (*6*). That author stated that the number of observable oil glands per petal increased significantly from 130 to 185 between the large bud stage and open flowers, noting that this was probably due to swelling rather than initiation of new oil glands. MacTavish (*6*) also observed large increases in both extract yield and the percentage of volatiles in the extract between the large bud stage and open flowers. The observation by MacTavish and Menary (*9*) that the β-ionone contents as a percentage of fresh flower weight were similar at the large bud and open flower stages was also consistent with the current study.

The observations also allowed consideration of the mechanism for the postharvest increases in β-ionone (up to approximately 25%) demonstrated by MacTavish and Menary (*11*) during incubation of the harvested flowers. In this regard ongoing carotenoid metabolism and site-specific cleavage in the 9,10-position may have the potential to result in the large increases observed in incubated flowers after harvest. This is supported by the additional observations of MacTavish and Menary (*11*) that the largest postharvest increases were achieved with undamaged open flowers in a well-oxygenated environment. These factors all indicated that there is ongoing metabolic activity during the postharvest incubation process. The use of a postharvest process may be a way of accumulating the newly formed β-ionone and other volatiles rather than allowing them to be lost to the atmosphere, following emission from the free-standing flower, and this rationale was discussed by MacTavish and Menary (*11*).

In summary, the biosynthetic flux in boronia is directed toward biosynthesis of β-carotene with subsequent site-specific cleavage of this molecule in the 9,10-position. Future experimental work will be necessary to confirm that the postharvest increase in β-ionone is due to ongoing carotenoid metabolism and subsequent site-specific cleavage. Examination of the extent of xanthophyll biosynthesis and cleavage, based on the increase in the levels of hydroxy 10′-apocaroten-10′-oic acid, may provide insight into the extent and relevance of these pathways in boronia. In addition, the extent of glycosylation of hydroxylated C-13 norisoprenoids should be examined as these compounds are found in a wide range of plants (*41*), and the potential exists for them to be an important source of flavor and aroma compounds in the extract.

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