One Tangerine/Grapefruit Hybrid (Tangelo) Contains Trace Amounts of Furanocoumarins at a Level Too Low To Be Associated with Grapefruit/Drug Interactions

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ABSTRACT: Tangelos are citrus hybrids with grapefruit and tangerine parentage and are a significant crop in Florida, marketed as specialty fruit. Because tangelos contain genetic material from grapefruit, there is the question of whether or not they contain furanocoumarins and exhibit an interaction with the drugs that have been associated with the grapefruit-drug interaction. Authentic samples of 13 tangerine-grapefruit hybrids (tangelos), 1 tangerine-orange hybrid, and 1 mandarin tangerine from Florida were obtained and screened for furanocoumarin monomer and dimer contents. None of the tangerine or tangerine hybrids were found to contain any furanocoumarins except for the K-Early variety, which contained trace amounts of 6,7-dihydroxybergamottin (0.028 ppm ± 0.0034), bergapten (0.011 ppm ± 0.005), and bergamottin (0.025 ppm ± 0.011). These amounts are low and insignificant in that they are not at levels that would significantly inhibit CYP3A4 enzyme and affect absorption of drugs metabolized by this enzyme. This information presents strong evidence that the commercial tangerine and tangelo varieties grown in Florida are unlikely to cause any interaction with the drugs affected by grapefruit.

Keywords: CYP3A4, furanocoumarin, psoralen, tangelo, tangerine, interaction

Introduction

Grapefruit juice, when consumed with some orally administered medications, has been shown to increase the bioavailability of certain drugs. Tangelos are a citrus hybrid cross containing genetic material from both grapefruit (*Citrus paradisi* Macf.) and tangerine (*Citrus reticulata*). The amount of genetic material from grapefruit in tangelo hybrids varies (Table 1) because of the extensive cross-breeding done for development of varieties with desired characteristics. Because tangelos contain genetic material from grapefruit, there is a question of whether these hybrids contain any of the same components found in grapefruit that interact with drugs metabolized by intestinal CYP3A enzymes and primarily the CYP3A4 isozyme.

It is now fairly well accepted that the likely components in grapefruit completely or largely responsible for CYP3A enzyme inhibition are naturally occurring furanocoumarin monomers and dimers (Dresser and others 2000; Kane and Lipsky 2000). Recently, 2 clinical trials were performed that compared a control grapefruit juice with a grapefruit juice from which the furanocoumarins had been selectively removed (>99%) on the absorption of felodipine (Paine and others 2003) and cyclosporine (Paine and others 2004). Felodipine is a calcium channel blocker medication used in the treatment and control of high blood pressure, whereas cyclosporine is an immunosuppressive medication used to reduce organ rejection in transplant patients; and both have been demonstrated to whow increased absorption when consumed with grapefruit juice. Although the control grapefruit juice significantly increased absorption of both drugs, the treated juice from which the furanocoumarins had been removed did not show any increased absorption compared with orange juice. Orange juice does not interact with any of the drugs associated with the grapefruit-drug interaction phenomenon.

The structures shown in Figure 1 have been identified as inhibitors of CPY3A4 enzyme activity; they include both furanocoumarin monomer and dimer compounds. The dimer compounds identified are composed of 2 furanocoumarins either linked by joining the terpene side chains (tail-to-tail) or by linking the terpene side chain to the ketone oxygen on the aromatic ring (head-to-tail). The Japanese researchers (Fukuda and others 1997; Guo and others 2000; Tassaneeyakul and others 2000) isolated and identified only 1 dimer with a head-to-tail linkage, whereas Harris (1999) isolated and identified several naturally occurring head-to-tail furanocoumarin dimers from grapefruit juice, calling them orthospiroesters (OSEs). Using the screening method developed by Harris (1999), with modifications to provide a better separation, we have identified 6 OSEs in grapefruit products using UV absorbance and mass spectral data. Tangelos available commercially as well as several not in production were screened for furanocoumarin content to determine whether they are likely to exhibit a drug interaction such as that found with grapefruit products. Florida-processed grapefruit juice contains an average of 2 to 3 ppm 6,7-dihydroxybergamottin (DHB), 3 to 4 ppm bergamottin, and much smaller amounts of the dimer compounds (Widmer and Haun 2005).

Materials and Methods

Chemicals

Bergamottin (5-geranoxypsoralen) and bergapten (5-methoxypsoralen) were purchased from Extrasynthase (Genay, France). The DHB was a gift from Antonio Montanari, Florida Dept. of Citrus.

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The DHB was obtained by extraction and purification by preparative high-performance liquid chromatography (HPLC) from grapefruit peel because it is not available commercially. The polymethoxylated flavones tangeretin, nobiletin, iso-sinensetin, sinensetin, tetramethyl-O-scutellarein, hexamethyl-O-quercetagetin, and 3,5,6,7,8,3′,4′-heptamethoxyflavone used were previously isolated and purified from Dancy tangerine oil (Chen and others 1996). Water, ethyl acetate, and acetonitrile used were HPLC-grade.

Sample collection

K-Early fruit were obtained in October from a local grove in Tavares, Fla. The fruit collected were verified to be K-Early by performing a flavonoid profile analysis (Rouseff and others 1987). One tangerine, 14 tangerine hybrids (Table 1), and grapefruit (for comparison) were obtained in November and December from the Florida Dept. of Plant Industries, Citrus Budwood Registry, to give a total of 15 specialty fruit varieties collected. For each variety, 2 fruit from the east, west, north, and south sides of the tree were collected for a total of 8 fruit per variety. Additionally, because the K-Early variety was found to contain trace amounts of furanocoumarins, a sample of K-Early juice was obtained from a local processing plant in early January and verified as before to be K-Early by the flavonoid profile.

Sample preparation

Four fruit from each variety were peeled, and all mature seeds carefully removed to minimize juice loss, leaving the fruit portion typically consumed. Fruit sections were then blended in a Cuisinart (East Windsor, N.J., U.S.A.) food mill to give a puree. A 20.8-g amount (equivalent to 20 mL) of fruit or juice was then weighed and extracted with 20 mL of ethyl acetate for 15 min with agitation. Samples were weighed to avoid problems associated with pipetting liquids containing pulp and other particulates. Layers were separated by centrifugation, and the ethyl acetate was decanted. Samples were extracted 3 more times using 10 mL of ethyl acetate and agitated for 15 min. The ethyl acetate extracts were combined and concentrated to near dryness in a rotary evaporator at 25 °C. Extracts were then transferred to a 5-mL volumetric flask and brought to volume with acetonitrile, filtered through a 0.45-μm nylon filter, and analyzed immediately or stored at 4 °C overnight before analysis. All fruit variety samples were extracted in duplicate.

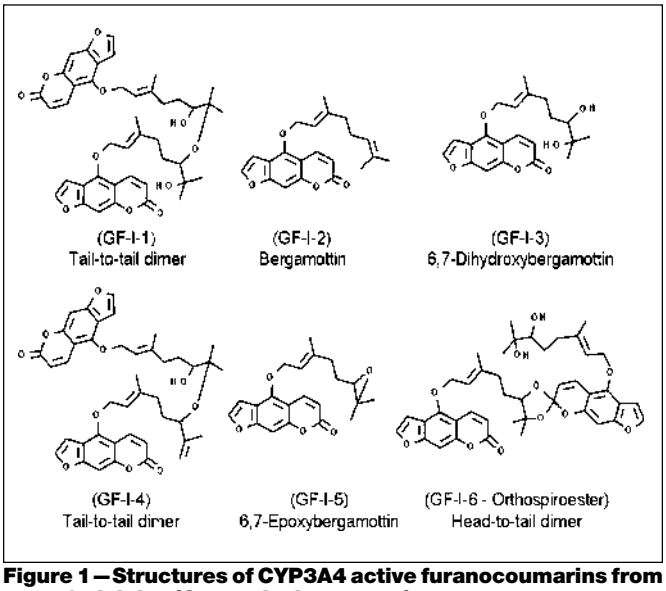

Figure 1—Structures of CYP3A4 active furanocoumarins from grapefruit juice (Guo and others 2000)

Table 1—Tangerine and tangerine hybrid varieties tested, parental cross, grapefruit parentage, and furanocoumarins detected[a]

Variety	% Grapefruit	Furano-coumarins
Murcott (Tangerine × Orange)	0%	ND
Dancy	0%	ND
Fallglo (Bower × Temple)	12.5%	ND
Ambersweet ([Orlando × Clementine] × Orange)	12.5%	ND
Robinson (Clementine × Orlando)	25%	ND
Lee (Clementine × Orlando)	25%	ND
Nova (Clementine × Orlando)	25%	ND
Osceola (Clementine × Orlando)	25%	ND
Page (Clementine × Minneola)	25%	ND
Sunburst (Robinson × Osceola)	25%	ND
Minneola (Honeybelle) (Dancy × Duncan)	50%	ND
Orlando (Lake) (Dancy × Duncan)	50%	ND
Seminole (Dancy × Duncan)	50%	ND
K-Early (Dancy × Bowen)	50%	TRACE
Sampson (Dancy × unnamed grapefruit)	50%	ND

[a]ND = not detected.

HPLC analysis

Samples were analyzed using a Thermo Electron Corp. (San Jose, Calif., U.S.A.) Spectrasystem comprising a P4000 pump, AS3000 autosampler with oven and sample tray temperature control, P6000 light pipe photodiode array detector (PDA), Chromquest V3.0 system control, and data analysis software. Samples were analyzed in duplicate using 10-μL injections onto a Waters (New Bedford, Mass., U.S.A.) YMC J-Sphere M80 3 × 250 mm 4-μm C-18 column. Two gradient profiles were used (Table 2) that were modified from the method described by Harris (1999). Data were collected using the PDA from 200 to 360 nm, a 1-s rise time, and a 5-nm window. The limit of detection for the furanocoumarin components was 10 ppb measured as bergamottin at 310 nm.

Results and Discussion

Chromatographic traces comparing extracts prepared from the edible portions of K-Early tangelo to Duncan grapefruit (Figure 2a) and Fallglo tangelo to ruby red grapefruit (Figure 2b) are shown. These are representative tracings of 2 tangelos with a grapefruit trace for comparison. Both furanocoumarin and polymethoxylated flavone peaks are identified because the polymethoxylated flavones have similar retention characteristics to DHB and bergapten. The 2 gradients were used to optimize the separation of earlier (gradient A) and later (gradient B) eluting peaks, although gradient B provided the better overall separation of the furanocoumarins and polymethoxylated flavones. Bergamottin, DHB, bergapten, and the polymethoxylated flavones were identified on the basis of a comparison of retention time and UV absorbance data with those of authentic standards. Identification of OSEs, a tail-tail furanocoumarin dimer (TTD), and epoxybergamottin were based on comparisons of mass spectral and UV information from published data (Fukuda and others 1997; Harris 1999; Widmer and Haun 2005). Peaks eluting before 15 min are primarily flavonoid in nature. Several other peaks in the tangelo varieties were not conclusively identified, but they did not contain spectral data characteristic of furanocoumarins.

Tangelos can resemble a tangerine, orange, or grapefruit in physical and flavor attributes. The genetic origin, percent content of the genetic material from grapefruit, and results for the presence of furanocoumarins for all the tangelo varieties tested are listed in Table 1. Murcott and Dancy tangerines, 2 varieties that do not con-

Table 2—High-performance liquid chromatography (HPLC) gradient profiles

Gradient A			Gradient B		
Time (min)	% Water	% Acetonitrile	Time (min)	% Water	% Acetonitrile
0	80	20	0	70	30
10	60	40	5	70	30
20	60	40	30	20	80
45	20	80	40	20	80
50	5	95	41	5	95
55	5	95	50	5	95
56	80	20	53	70	90
65	80	20	60	70	30

tain any genetic material from grapefruit and are popular commercial varieties grown in Florida, were also tested. The origin of Murcott is believed to be a tangerine and orange cross with the exact parentage unknown. Dancy is a true mandarin tangerine. The main tangelos grown in Florida for the specialty fruit market include Fallglo, Sunburst, Minneola, Robinson, and Orlando varieties. Page, Nova, Osceola, and Lee tangelos have little commercial importance, whereas the varieties Sampson and Seminole are not produced commercially. The variety Ambersweet, officially classified as an orange, is an early-season hybrid variety with good fruit and juice color characteristics. Its value is mainly to improve color in early season orange juice. The K-Early variety, having the appearance of an orange and flavor character of a grapefruit, has been grown and marketed to a limited extent as an early-season grapefruit. Due to the poor fruit quality of K-Early, production and use has declined over the years. Production decreased from 5000 tons used during the 1999-2000 season to less than 1400 tons used during the 2001-2002 fruit season, the last year The Florida Dept. of Agriculture and Consumer Services (2005) reported production figures for K-Early variety. Commercial production in Florida was reported to be less than 675 tons during the 2003-2004 season (private communication) compared with production figures of greater than 45000 tons of all other tangelos, 292000 tons of tangerines, 1.7 million tons of grapefruit, and 10.9 million tons of oranges (FDACS 2005).

Of the tangelos tested, only K-Early and Sampson varieties resemble grapefruit in flavor and appearance. Both these varieties contain the bitter flavonoids naringin and neohesperidin, which

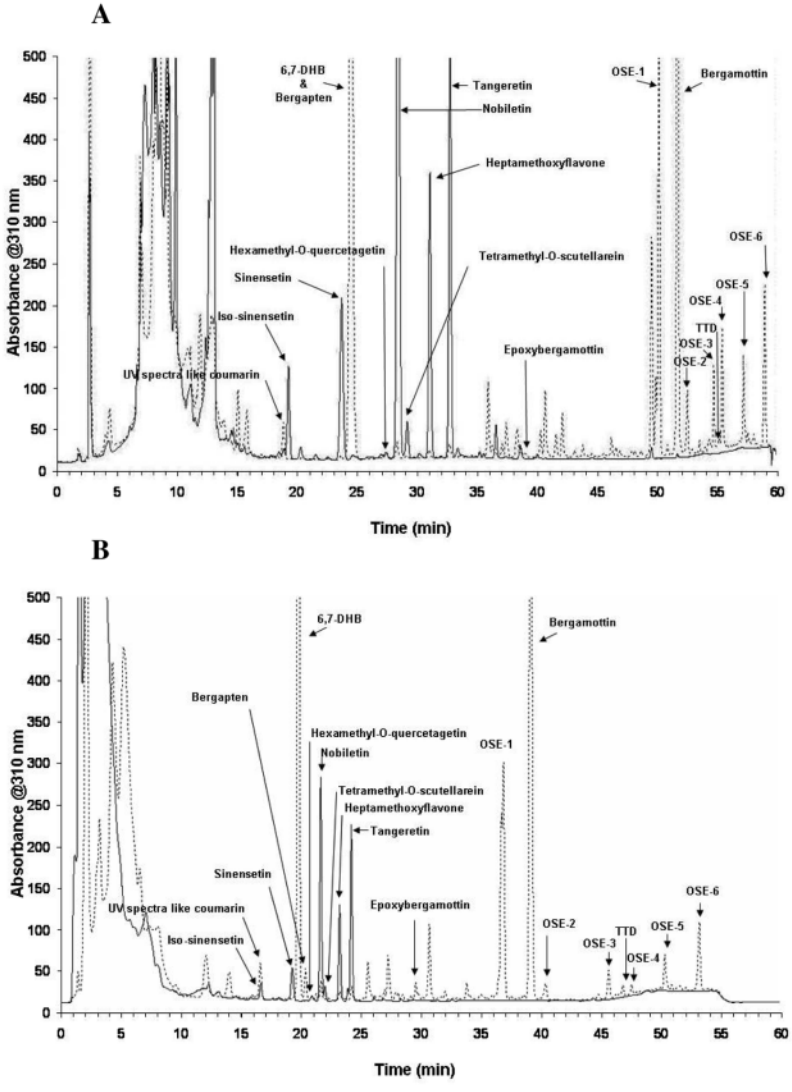

Figure 2—Chromatographic separations at 310 nm comparing (a) extracts of edible portion (juice sacs and segment membranes) from fresh K-Early (solid line) and Duncan grapefruit (dashed line); (b) extracts of the edible portion from fresh Fallglo tangelo (solid) and ruby red grapefruit (dashed line). Furanocoumarin peaks in grapefruit are identified and compared with polymethoxylated flavone peaks present in both tangelos and grapefruit. Conditions are as described in the text. OSE = orthospiroester furanocoumarins; TTD = tail-tail furanocoumarin dimer.

contribute to the characteristic grapefruit flavor. None of the other 12 tangelos contain these bitter flavonoids. Of all the varieties, K-Early and Sampson would be expected to be the most likely to contain furanocoumarins because they not only contain 50% of their genetic material from grapefruit, but also resemble grapefruit. No furanocoumarin components were detected in the Sampson variety, and the K-Early variety collected in October contained trace amounts of DHB (0.029 ppm ± 0.0018), bergapten (0.015 ppm ± 0.0002), and bergamottin (0.015 ppm ± 0.0007). Because the K-Early sample collected in October contained trace amounts of furanocoumarins, a sample of K-Early juice was obtained from a local processor in early January. This sample represented most of the K-Early crop grown during the 2003-2004 season. The K-Early juice sample (verified to be K-Early by flavonoid profile) also contained trace amounts of DHB (0.034 ppm ± 0.0030), bergapten (0.007 ppm ± 0.0014), and bergamottin (0.035 ppm ± 0.0007). No furanocoumarin dimers were detected in either K-Early sample. The total of the 3 furanocoumarins detected in K-Early is less than 0.2 μM, a level too low to significantly interact with intestinal CYP3A4 enzyme. Evidence that these amounts are too low to have any interaction comes from the 2 recent clinical trials performed by Paine and others (2003, 2004) showing that when furanocoumarins are selectively removed from grapefruit juice, the drug interaction is eliminated. The treated grapefruit juice tested, which had >99% of the furanocoumarins removed, contained a residual furanocoumarin amount that was more than twice the amount contained in the K-Early fruit. None of the other 13 tangelo varieties contained detectable amounts of any furanocoumarin or furanocoumarin dimer compounds.

Conclusions

These results are reassuring because it means that none of the tangelos are likely to exhibit the drug interaction problems found with grapefruit. The information is important to growers and consumers of these specialty fruit because it lends evidence that tangerines and tangelos grown commercially in the United States are unlikely to cause any problems when consumed with drugs metabolized by the intestinal CYP3A4 enzyme.

Acknowledgments

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Dept. of Agriculture.

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