Evaluation of Auraptene Content in Citrus Fruits and Their Products

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Auraptene quantities in Tanaka's 77 *Citrus* species (including 14 varieties and cultivars), 5 *Fortunella* species, one *Poncirus* species, 27 hybrids between *Citrus* species, and 51 intergeneric hybrids between *Citrus* and *Poncirus* have been evaluated. The genus *Citrus* has been divided into eight groups. Auraptene is found in all of the species of Cephalocitrus group, a part of the species of Aurantium group, and most of the species of Osmocitrus group. The *Citrus* species contain a small amount of auraptene in the juice sacs compared with in the peels except for Henka mikan (*C. pseudo-aurantium*), Ichang lemon (*C. wilsonii*), and a Hassaku (*C. hassaku*)-pummelo hybrid (Okitsu No. 39), which contain large quantities of auraptene in their juice sacs (0.23, 0.52, and 0.14 mg/g, respectively). The Hong Kong wild kumquat (*F. hindusii*) alone contains auraptene in *Fortunella* species. All of the *Citrus*-trifoliate orange (*P. trifoliata*) hybrids as well as the trifoliate orange contain a large quantity of auraptene in both the peel (16.57−0.51 mg/g) and the juice sac (10.32−0.15 mg/g). These hybrids are almost inedible. The Iyo (*C. iyo*)-trifoliate orange hybrid (IyP269) is edible and contains auraptene in the peel (1.49 mg/g) and in the juice sac (1.73 mg/g). *Citrus* fruit products, for example, brand-named grapefruit juice and marmalade, retain about 0.1 mg and 0.3 mg/100 g of auraptene, respectively.

Keywords: *Auraptene; Citrus; Fortunella; Poncirus; hybrid; peel; juice sac; fruit product; HPLC*

INTRODUCTION

Numerous studies indicate that increasing the consumption of fruits and vegetables reduced the risk of cancer (Steinmetz and Potter, 1991; Block et al., 1992). It has been suggested that particular phytochemicals contained in fruits and vegetables inhibit or reverse the process of cancer development (Steele et al., 1994; Tanaka, 1994; Sharma et al., 1994). Citrus peels contain a number of coumarins that possess mevalonate-derived side chains with various oxidation levels. Auraptene is a simple coumarin bearing a geranyloxyl moiety at C-7. Recent studies revealed that auraptene inhibits tumor promotion in ICR mouse skin (Murakami et al., 1997), growth of colonic aberrant crypt foci in rats (Tanaka et al., 1997), oral carcinogenesis in rats (Tanaka et al., 1998), and large bowel tumorigenesis in rats (Tanaka et al., 1998). These inhibitory activities correspond to the suppression of superoxide generation (Murakami et al., 1997) and lipid peroxidation and the induction of phase II drug-metabolizing enzymes (Tanaka et al., 1998). These facts suggest that auraptene is a promising cancer-preventive component contained in citrus fruits.

Auraptene has been found in citrus plants such as the sour orange (*C. aurantium*), Natsudaidai (*C. natsudaidai*), grapefruit (*C. paradisi*), and trifoliate orange (*Poncirus trifoliata*) as well as in some species belonging to Rutales (Gray and Waterman, 1978; Gray, 1983). The juice oil of Hassaku (*C. hassaku*) is a rich source of coumarins, and auraptene comprises more than 70% of the oil's constituents (Masuda et al., 1992). Auraptene is considered to be a major coumarin of citrus plants. The presence of auraptene exhibiting its cancer-preventing property is regarded as a character incidental to citrus fruits.

The evaluation of auraptene content in citrus fruits gives us some information about cancer prevention based on dietary habits. Tsuchida et al. (1997) analyzed 34 chemical constituents in peels of 35 citrus plants, but auraptene was not identified. Tanaka (1969) enumerated 159 species of the genus *Citrus*. Data on the auraptene contents in citrus fruits, especially in the edible portion (juice sac), remain incomplete. This report reveals the auraptene contents in 77 *Citrus* species (including 11 varieties), five *Fortunella* species, and one *Poncirus* species. The citrus breeding has produced a lot of hybrid progenies, most of which have been screened out because of characteristics contrary to general breeding aims. The auraptene content in the hybrid progenies is unknown. The enrichment of phytochemicals such as auraptene is usually not included in the breeding aims. There is a good possibility that hybrids remarkably enriched with auraptene are picked out from the hybrid progenies. The inheritance of auraptene accumulation is a necessary feature in breeding focused on the enrichment of auraptene contents. Thus, we have examined the fluctuation of auraptene contents in 78 hybrids produced from parent fruit containing auraptene.

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10.1021/jf9905525 CCC: $19.00
Published on Web 04/14/2000

Some citrus fruits are used in making preserves, such as marmalade, jam, and crystallized fruit, and for juice and culinary purposes (seasoning, condiments, and dressings). Since these fruit products are considered to be a dietary source of auraptene, we have evaluated the auraptene content in some of these products.

MATERIALS AND METHODS

Plant Materials. Citrus plants, listed in Tables 1, 2, and 3, were selected from germplasm reserved in the Department of Citriculture at the National Institute of Fruit Tree Science. Fruits were harvested from each citrus tree in November 1996, December 1997–January 1998, or September–October 1999. For all the fruit studied, the whole fruit was roughly cut, frozen at −80 °C, freeze-dried, and then divided into peel (epicarp and mesocarp), juice sacs, endocarp, and seeds. In this report, peel and juice sacs were analyzed. The samples were pulverized with a cutter and stored at −30 °C for analysis.

Citrus Fruit Products. Brand-named citrus fruit products, including orange marmalade, grapefruit juice, orange juice, ponzu, and yuzumiso, were selected and purchased for auraptene analysis. The orange marmalade products in the Japanese market are made from a mixed peel, such as that of sweet oranges (*C. sinensis*) and Natsudaidai (*C. natsudaidai*). The juice products were single-strength, diluted from juice concentrate. Ponzu and yuzumiso are Japanese traditional condiments. Ponzu is a mixture of soy sauce and the extract of sour and flavorful citrus fruits such as Yuzu (*C. junos*) and Kabosu (*C. sephaerocarpa*). Yuzumiso is made from Yuzu extract and bean paste miso.

Standard Sample. Auraptene was obtained from the fresh peel of Natsudaidai (*C. natsudaidai*) and identified by comparing it with the published data (^{1}H and ^{13}C NMR) and by direct comparison with the data of an authentic sample (HPLC, UV, and first atom bombardment MS). Auraptene was recrystallized from ethanol/water solution and dried in vacuo at 60 °C.

Preparation of Analytical Samples. Samples (1.000 g) were ground in acetone (15.0 mL) by using a mixer (Kinematica, Switzerland) for 3 min. The mixing generator was rinsed with ethanol (5 mL × 2). The acetone and the ethanol solutions were combined and filtered. The residue on the filter paper was washed with acetone (50 mL). The filtrate was concentrated, dissolved in methanol/acetone (1:1), and adjusted to 5.0 mL in a volumetric flask. One hundred grams of juice product was centrifuged at 10 000 rpm for 5 min. The supernatant was extracted with ethyl acetate (100 mL, three times). Ethanol (50 mL) was added to the precipitate, which was then vortex-mixed for 1 min and centrifuged again. The ethyl acetate extract and the second supernatant were combined and filtered through paper. After removal of the solvent from the filtrate in vacuo, the residue was dissolved in methanol/acetone (1:1) and filled up to 5.0 mL.

Marmalade (20.0 g) and yuzumiso (20.0 g) were mixed with water (150 mL) and homogenized by using a mixer for 3 min. The slush was extracted with ethyl acetate (150 mL × 3). The extracts were combined, filtered, and concentrated in vacuo. The residue was dissolved into methanol/acetone (1:1) and adjusted to 5.0 mL in a volumetric flask. Ponzu (10 mL), which was shaken vigorously before sampling in order to disperse oil into the aqueous phase, was subjected to an ODS short column (Toyopak ODS M, Tosoh, Japan). The column was washed with water and eluted with methanol/acetone (50:50). The eluant was concentrated in vacuo, dissolved in methanol/acetone (50:50), and adjusted to 2.0 mL in a volumetric flask. The solutions were filtered through a PTFE filter (0.20 μm) and subjected to an HPLC analysis.

HPLC Analysis. The analysis was carried out under the following conditions: column, Hypersil ODS column (5 μm, 125 mm × 4 mm i.d., Hewlett-Packard); injection volume, 5.0 μL; column temperature, 40 °C; flow rate, 1.0 mL/min; solvent system, methanol/water (75:25); detection, UV absorption at 325 nm. The auraptene quantities were estimated by the absolute calibration method. The peak for auraptene was identified by its UV spectrum obtained by a diode-array detector. HPLC profiles of authentic auraptene and juice sac extracts of Ichang lemon (*C. wilsonii*), trifoliate orange (*P. trifoliata*), and hybrid IyP269 are shown in Figure 1.

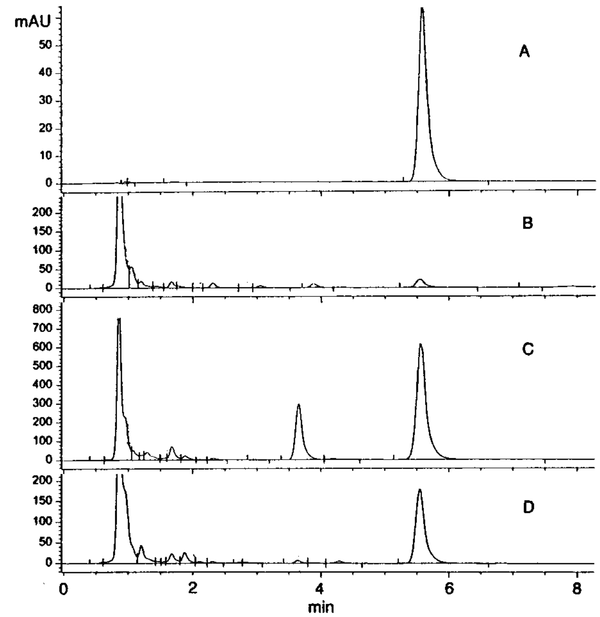

Figure 1. HPLC profiles of juice sac extracts (A) auraptene, (B) Ichang lemon (*Citrus wilsonii*), (C) trifoliate orange (*Poncirus trifoliate*), and (D) IyP269; a hybrid between the iyo (*C. iyo*) and trifoliate orange.

RESULTS AND DISCUSSION

Auraptene Content in 83 Citrus Fruits. The genus *Citrus* has undergone natural crossing during a long period of cultivation and is composed of many natural hybrids (Hodgson, 1967; Tanaka, 1946, 1948, 1980). It is generally accepted that citron (*C. medica*), pummelos (*C. grandis*, *C. maxima*), and mandarins (*C. reticulata*) are probably true species and all other species are thought to be of hybrid origin (Barrett and Rhodes, 1976). There are two major systematics of the genus *Citrus*. Swingle's systematics (Swingle and Reece, 1967) includes 16 species. The other systematics, proposed by Tanaka, consists of 159 species (Tanaka, 1969). Tanaka's systematics does not discriminate between the true species and the species originating from natural hybrids and lists the many relatives in order of morphological resemblance. In this paper, we have used Tanaka's systematics.

The auraptene contents in fruits of *Citrus*, *Fortunella*, and *Poncirus* species are summarized in Table 1. According to Tanaka's systematics, the genus *Citrus* has been subdivided into eight groups, Papeda, Limonellus, Citrophorum, Cephalocitrus, Aurantium, Osmocitrus, Acrumen, and Pseudofortunella. All the species of Cephalocitrus contain auraptene in their peels. Auraptene was not detected in the species belonging to Limonellus, Acrumen, and Pseudofortunella. The species of Citrophorum, Aurantium, and Osmocitrus are divided further into groups containing or lacking auraptene. Auraptene is found in the juice sacs of the species that contain auraptene in the peel. There is less auraptene in the juice sacs than in the peels, except in the case of a unique species, the Ichang lemon (*C. wilsonii*).

Cephalocitrus includes pummelos (*C. grandis*) and pummelo relatives. We examined 18 species, including

Table 1. Auraptene Content in the Peels and Juice Sacs of Various Citrus Plants[a]

scientific name[b]	common name	mg/g (dry weight) peel	mg/g (dry weight) juice sac
Citrus			
Papeda			
C. hystrix	Purrut	tr[c]	tr
Limonellus			
C. latifolia	Tahiti lime	nd[d]	nd
C. bergamia	Bergamot	nd	nd
C. montana	Biroro	tr	na[e]
Citrophorum			
C. medica	Citron	nd	na
C. limon	Eureka lemon	nd	nd
C. limonia	Limonia	0.014	na
C. limetta	Sweet lemon	nd	nd
C. jambhiri	Rough lemon	tr	nd
C. meyeri	Meyer lemon	0.013	na
C. lumia	Lumie	tr	nd
Cephalocitrus			
C. grandis	Hirado buntan	0.048	0.007
	Mato buntan	0.074	0.004
	Banpei yu	0.093	0.030
	Kao Pan	0.227	0.012
	Kao Phuang	0.164	0.004
	Anseikan	0.189	0.023
	Egami buntan	0.012	0.013
	Shaten yu	0.100	0.006
C. truncata	Kaikokan	0.125	0.014
C. pseudogulgul	Shishi yuzu	0.091	0.009
C. paradisi	Marsh grapefruit	0.432	0.040
C. glaberrima	Kinukawa	0.408	tr
C. hiroshima	Hiroshima natsuzabon	0.244	0.040
C. hassaku	Hassaku	0.588	0.035
	Wako hassaku	0.810	0.049
	Nomabeni hassaku	1.149	0.055
C. iwaikan	Iwaikan	0.026	0.004
C. tengu	Tengu	0.004	0.047
Aurantium			
C. medioglobosa	Naruto	1.453	0.036
C. natsudaidai	Natsudaidai	0.586	0.022
	Kawano natsudaidai	1.044	0.061
C. ampullacea	Hyokan	0.493	0.032
C. sulcata	Sanbokan	tr	nd
C. aurantium	Sour orange	nd	nd
	Kabusu	nd	nd
	Zadaidai	nd	nd
	Bouquet de Fleurs	nd	nd
C. rokugatsu	Rokugatsu mikan	nd	nd
C. sinensis	Valencia orange	nd	nd
	Navel orange	tr	nd
C. sinograndis	Oto-mikan	0.560	0.016
C. tankan	Tankan	nd	nd
C. iyo	Iyo	0.014	0.002
C. tamurana	Hyuganatsu	nd	nd
C. ujyukitsu	Ujyukitsu	tr	nd
C. aurea	Kawabata	0.097	0.001
C. shunkokan	Shunkokan	0.006	0.003
Osmocitrus			
C. ichangensis	Ichang papeda	nd	nd
C. junos	Yuzu	0.376	0.055
C. hanaju	Hanayu	0.009	0.002
C. sudachi	Sudachi	0.004	0.003
C. inflata	Mochiyu	0.004	0.003
C. pseudo-aurantium	Henka mikan	1.127	0.237
C. wilsonii	Ichang lemon	0.094[f]	0.521[g]
C. sphaerocarpa	Kabosu	0.310	0.026
C. nippokoreana	Korai-tachibana	0.015	tr
Acrumen			
C. nobilis	King	nd	nd
	Kunenbo	nd	nd
C. unshiu	Satsuma mandarin	nd	nd
C. yatsusiro	Yatsusiro	nd	nd
C. keraji	Keraji	nd	nd
C. oto	Oto	nd	nd
C. reticulata	Ponkan	tr	nd
C. deliciosa	Mediterranean mandarin	nd	nd
C. tangerina	Dancy tangerine	nd	nd
C. clementina	Clementine	nd	nd
C. succosa	Jimikan	nd	nd
C. suhuiensis	Shikaikan	nd	nd
C. tachibana	Tachibana	nd	nd
C. erythrosa	Kobeni-mikan	nd	nd
C. kinokuni	Kishu-mikan	nd	nd
C. sunki	Sunki	nd	nd
C. tardiva	Giri-mikan	nd	na
C. depressa	Shiikuwasha	nd	nd
C. leiocarpa	Koji	nd	nd
Pseudofortunella			
C. madurensis	Calamondin	tr	nd
other			
C. halimii		tr	nd
Fortunella			
Protocitrus			
F. hindsii	Hong Kong wild kumquat	0.130	0.014
Eufortunella			
F. crassifolia	Meiwa kumquat	nd	nd
F. japonica	Marumi kumquat	nd	nd
F. margarita	Nagami kumquat	nd	nd
F. polyandra	Malayan kumquat	nd	nd
Poncirus			
P. trifoliata	Trifoliate orange	1.312	6.563

[a] Specimens were collected in November 1996, October 1997−Feburary 1998, and September−October 1999. [b] The nomenclature of the plants is mainly based on Tanaka's systematics. [c] tr, trace amount below 0.001 mg/g. [d] nd, not detected at a detection limit (0.0001 mg/g). [e] na, not analyzed. [f] SE = 0.054 (n = 3). [g] SE = 0.057 (n = 3).

9 varieties and cultivars. Pummelos (*C. grandis*) are self-incompatible and can easily be hybridized to give rise to many varieties. All the pummelo (*C. grandis*) varieties analyzed contain auraptene, but its content varies widely (0.012−0.227 mg/g in peels and 0.004−0.030 mg/g in juice sacs). Pummelo relatives of Cephalocitrus are thought to be hybrids between pummelos and other *Citrus* species and exhibit the characteristics of the pummelo predominantly. Grapefruit (*C. paradisi*) probably originated as a hybrid between the pummelo (*C. grandis*) and the sweet orange (*C. sinensis*). A cultivar of grapefruit, Marsh, contains 0.432 mg/g of auraptene in its peel. Hassaku (*C. hassaku*) is thought to be a hybrid between a pummelo (*C. grandis*) and a mandarin (*Citrus* species belonging to the Acrumen group). Three cultivars of Hassaku were analyzed. It is notable that the peel of Nomabeni hassaku, which is a bud mutation of Hassaku, contains a large amount of auraptene (1.149 mg/g) compared with the other cultivars.

Aurantium includes pummelo relatives, sour oranges (*C. aurantium*), sweet oranges (*C. sinensis*), and probably natural tangors [hybrids between mandarins (the species belonging to Acrumen) and sour orange (*C. aurantium*)]. Nineteen species, including 5 varieties and cultivars, were analyzed. Not all the species of Aurantium contain auraptene. Pummelo relatives of Aurantium, such as Natsudaidai (*C. natsudaidai*), sour oranges, and sweet oranges, probably originated from natural tangelos that are hybrids between pummelos (*C. grandis*) and mandarins. The Natsudaidai contains auraptene in its peel (0.586 mg/g), while the sour oranges (*C. aurantium*) and sweet oranges (*C. sinensis*) lack auraptene, though it was described in reviews that the sour orange contained auraptene (Gray and Waterman, 1978; Gray, 1983). The Natsudaidai exhibits characteristics of the pummelo. It is said that Kawano natsudaidai has originated from a limb sport. It contains a higher amount of auraptene (1.044 mg/g) than Natsudaidai.

The characteristics of sour oranges (*C. aurantium*) and sweet oranges (*C. sinensis*) are close to those of mandarins. The genetic relationships based on isoenzymatic systems indicated that *Citrus* species were divided into two main groups; one is the mandarin (*C. reticulata*) group and the other is the pummelo (*C. grandis*) group (Herrero et al., 1996). Sour oranges (*C. aurantium*) are combined with mandarins (*Citrus* species belonging to the Acrumen group) and are distinct from pummelos. Distribution of auraptene is coincident with the above relationships. Tankan (*C. tankan*) is thought to be a natural tangor. Mandarins and sour oranges, which are parental species of tangors, contain no auraptene. Hybrids between parents lacking auraptene probably contain no auraptene. Pummelos contain auraptene without exception. On the other hand, hybrids involving a pummelo parentage do not necessarily contain auraptene. The Naruto (*C. medioglobosa*) has abundant auraptene in its peel (1.453 mg/g). Though it exhibits the morphological characteristics between sour oranges and mandarins, its origin is unknown. The presence of auraptene strongly suggests that Naruto may involve a pummelo parent.

Osmocitrus are composed of the Ichang papeda (*C. ichangensis*), Yuzu (*C. junos*), and Yuzu relatives. We analyzed nine species. The peel of the Yuzu (*C. junos*), Henka mikan (*C. pseudo-aurantium*), and Kabosu (*C. sphaerocarpa*) contain auraptene in large quantities (0.376, 1.127, and 0.315 mg/g, respectively), while there is almost no auraptene in the peels of the Hanayu (*C. hanayu*), Sudachi (*C. sudachi*), and Mochiyu (*C. inflata*). It is notable that the Henka mikan (*C. pseudo-aurantium*) and Ichang lemon (*C. wilsonii*) contain a large amount of auraptene in their juice sacs (0.237 and 0.521 mg/g, respectively), which is different from the case of other *Citrus* fruits. The Ichang papeda (*C. ichangensis*) contains no auraptene in either the peel or the juice sac. The Yuzu (*C. junos*) probably originated from a natural hybrid between an Ichang papeda (*C. ichangensis*) and a mandarin (*C. reticulata*). Neither of the parents contains auraptene. The Ichang lemon (*C. wilsonii*) is thought to be a natural hybrid between an Ichang papeda and a pummelo (*C. grandis*). Pummelos and pummelo relatives accumulate a small amount of auraptene in their juice sacs. These facts suggest that the parental species of the Yuzu (*C. junos*) and Ichang lemon (*C. wilsonii*) differ from the hypothesized combinations. The same conclusion has been proposed on the basis of isozyme analysis (Hirai et al., 1986). Though the Ichang lemon (*C. wilsonii*) is not a commercial cultivar, its juice is just like that of the lemon, as the common name shows, and it has been used occasionally to make lemon pies. The juice of Ichang lemon contains 4 mg/100 g of auraptene, making it a good dietary source of auraptene.

The Acrumen group consists of mandarin-type citrus such as the Satsuma mandarin (*C. unshiu*). We analyzed 19 species, including one variety, out of 36 species listed by Tanaka. All of the species lacked auraptene in both the peel and the juice sac. Though the Satsuma mandarin has been a major citrus product in Japan, it is not a dietary source of auraptene. Pseudofortunella consists of only Calamondin (*C. madurensis*), which lacks auraptene. *C. halimii*, a recently described wild *Citrus* indigenous to peninsular Thailand and Malaya, is not included in Tanaka's systematics. This species contains no auraptene.

The genus *Fortunella* includes six species according to Tanaka's systematics. We collected and analyzed five of these species and found that of them, only the Hong Kong wild kumquat (*F. hindsii*) contains auraptene. This species is thought to be an autotetraploid and was separated from the others according to Tanaka's systematics. This classification is consistent with the distribution of auraptene.

The fruit of the trifoliate orange (*P. trifoliata*) contains a large amount of auraptene in both the peel and the juice sac. Auraptene content in the juice sac (6.563 mg/g) is higher than in the peel (1.132 mg/g). Coumarins are generally stored in the oil glands of the peel. The juice sacs of trifoliate oranges contain yellowish semisolid inclusions of acrid oil in the center. Auraptene content in the extracted inclusions increased to 50 mg/g in dried weight. This result indicates that the oil droplets contain auraptene.

Hybrids. The above results suggest the combination of parental citrus plants causes the hybrids to be enriched with auraptene. Pummelos (*C. grandis*), some pummelo relatives, Ichang lemon (*C. wilsonii*), and trifoliate orange (*P. trifoliata*) are appropriate parental citrus species when trying to obtain auraptene-rich hybrids. The auraptene contents in 27 hybrids involving parentage of pummelos or pummelo relatives were confirmed, as shown in Table 2. Crosses among pummelos may yield hybrids with higher auraptene content. For example, the Hayasaki and Benimadoka originated from a cross between the Mato and Hirado buntan. The parents contain lower amounts of auraptene (0.047 and 0.048 mg/g, respectively) among pummelos. The auraptene contents in the peel of the hybrids (0.456 and 0.312 mg/g, respectively) are higher than that in the parents. Hybridization between the Hassaku (*C. hassaku*) and the Hirado buntan (*C. grandis*) gave rise to good cultivars, such as the Yellow pummelo and the May pummelo, which contain large amounts of auraptene in their peel (0.169 and 0.342 mg/g, respectively).

Though not a commercial cultivar, Okitsu No. 39 has rather good properties of fruit. It is a noteworthy hybrid because it has a much higher level of auraptene (0.147 mg/g) in its juice sac compared with that of its parent fruit. The reason for the high level of auraptene in the juice sac is not known. The moisture content of the juice sac is 85−90 g/100 g. As a comparison, eating 100 g of fresh grapefruit corresponds to taking about 0.4−0.6 mg of auraptene, while eating a similar amount of Okitsu No. 39 supplies at least 1.5 mg.

Crosses between Hassaku (*C. hassaku*) and the species absent in auraptene such as the Dancy tangerine (*C. tangerina*) and the Satsuma mandarin (*C. unshiu*)

Table 2. Auraptene Content in the Peels and Juice Sacs of Hybrids between *Citrus* Species[a]

seed parent × pollen parent	common name or tentative code	mg/g (dry weight)	
		peel	juice sac
C. grandis "Mato buntan" × *C. sulcata*?	Tanikawa buntan	0.031	tr[b]
C. grandis "Mato buntan" × *C. grandis* "Hirado buntan"	Hayasaki	0.426	0.015
C. grandis "Mato buntan" × *C. grandis* "Hirado buntan"	Benimadoka	0.312	0.021
C. hassaku × *C. grandis* "Hirado buntan"	Yellow pummelo	0.169	0.009
C. hassaku × *C. grandis* "Hirado buntan"	May pummelo	0.342	0.039
C. hassaku × *C. grandis* "Hirado buntan"	Okitsu No. 39	0.105	0.147
C. hassaku × *C. natsudaidai*	Summer fresh	0.348	0.022
C. hassaku × *C. tangerina*	Okitsu No. 26	nd[c]	nd
C. hassaku × *C. iyo*	Okitsu No. 40	nd	nd
C. unshiu × *C. hassaku*	Sweet spring	nd	nd
Sweet spring × *C. hassaku*	Okitsu No. 34	0.006	0.067
Sweet spring × *C. hassaku*	Okitsu No. 37	0.012	tr
Okitsu No. 37× Lee[d]	D396	nd	nd
Sweet spring × *C. hassaku*	Okitsu No. 38	0.041	nd
Sweet spring × *C. hassaku*	D158	0.068	0.084
Sweet spring × *C. hassaku*	D161	2.096	0.096
Sweet spring × *C. hassaku*	D177	0.476	0.068
Sweet spring × *C. hassaku*	D180	0.004	nd
Sweet spring × *C. hassaku*	D181	1.893	0.005
Sweet spring × *C. hassaku*	D226	0.002	0.002
Sweet spring × *C. hassaku*	D234	0.005	0.013
Sweet spring × *C. hassaku*	D241	0.829	0.035
Sweet spring × *C. natsudaidai* "Kawano"	D301	0.003	nd
Sweet spring × *C. sinensis* "Trovita"	Okitsu No. 46	nd	nd
Sweet spring × Lee	D395	nd	nd
C. unshiu × *C. hassaku*	Okitsu No. 42	nd	nd
C. grandis × *C. paradisi*	Oroblanco[e]	0.421	0.016

[a] Specimens were collected in December 1997 and January 1998. [b] tr, trace amount below 0.001 mg/g. [c] nd, not detected at a detection limit (0.0001 mg/g). [d] *C. clementina* × (*C. paradisi* Duncan × *C. tangerina*). [e] Specimen on the market (as of December 1997).

have given rise to hybrids that lack auraptene accumulation. Sweet spring is a cultivar produced by crossing the Satsuma mandarin (*C. unshiu*) and the Hassaku (*C. hassaku*). Hybridization between Sweet spring and Hassaku (*C. hassaku*) has afforded a considerable number of backcrossed hybrids. We analyzed 11 back-crossed hybrids. Four hybrids contain more than 0.1 mg/g of auraptene and four contain less than 0.01 mg/g. While Sweet spring lacks auraptene, the back-crossed hybrids exhibit segregation in regard to auraptene accumulation. The occurrence of the segregation can be accounted for by heterozygosity of pummelos and pummelo relatives for a dominant allele corresponding to auraptene accumulation.

The juice sac of the trifoliate orange contains auraptene in very large quantities. Unfortunately, it is completely inedible because of a characteristic unpleasant smell and taste. It is probable that hybrids having juice sacs high in auraptene content are produced by crosses between *Citrus* species and trifoliate orange (*P. trifoliata*). Since *Citrus*-trifoliate orange hybrids are cold-hardy and important as rootstock, many hybrids have been produced. Citranges [trifoliate orange−sweet orange (*C. sinensis*) hybrids], citrumelos [trifoliate orange−grapefruit (*C. paradisi*) hybrids], and citrandarins [trifoliate orange−mandarin (the species belonging the Acrumen group) hybrids] are well-known hybrids. We have a lot of intergeneric hybrids between *Citrus* species as the seed parent and the trifoliate orange as the pollen parent. We analyzed 51 hybrids, as shown in Table 3. All of these hybrids contained auraptene in large quantities, with contents in the peels of the hybrids generally higher than that in the parents. The hybrid SgP304, which is obtained by crossing a Satsuma mandarin (*C. unshiu* "Suzuki-wase") and a trifoliate orange, contained 16.571 mg/g of auraptene in its peel, the highest auraptene content of all the hybrids we analyzed. The juice sac of a hybrid (JP246) between the Yuzu (*C. junos*) and the trifoliate orange contained a fairly large amount (10.321 mg/g) of auraptene. Almost all of the hybrids analyzed contained lower amounts of auraptene in their juice sacs compared with the parents, however.

The hybrids including trifoliate orange as a parent mostly have an unacceptable smell and are inedible. Crossing Iyo (*C. iyo*) and trifoliate orange (*P. trifoliata*) has given rise to some edible hybrids, however. The hybrid IyP269 is completely free from the unpleasant smell associated with many of these hybrids. It has low °Brix (9.6) and is very sour. We can utilize it as a sour citrus and for making preserves, such as marmalade, crystallized fruits, and liquors. Although the auraptene contents of IyP269 are not very high compared to that of the other hybrids analyzed, the contents in the peel and juice sac are 1.494 and 1.736 mg/g, respectively. Auraptene content of IyP269 in the juice is 19 mg/100 g.

Crosses between Satsuma mandarins (*C. unshiu*) and trifoliate orange (*P. trifoliata*) have yielded unilaterally high auraptene content in the resulting hybrids. A back-cross between Hassaku (*C. hassaku*) and the hybrid MtWP400 produced hybrids lacking auraptene [H(MtWP400)56, 0.004 mg/g in peel and not detected in the juice sac] and containing auraptene [H(MtWP400)-59, 0.733 mg/g in peel and 0.039 mg/g in the juice sac]. These results suggest that auraptene accumulation of the intergeneric hybrids is probably controlled by a dominant allele and that the trifoliate orange (*P. trifoliata*) is homozygous for the allele. Combination of a citrumelo [trifoliate orange−grapefruit (*C. paradisi*) hybrid] with a sweet orange (*C. sinensis*) yields the edible hybrid US119 (Barrett, 1990). The taste of US119 is similar to that of the sweet orange, but it has almost no juice sac auraptene (0.003 mg/g). Crosses between Kiyomi, a hybrid of the Satsuma mandarin (*C. unshiu*) and the sweet orange (*C. sinensis*), and the intergeneric

Table 3. Auraptene Content in the Peels and Juice Sacs of Hybrids between *Citrus* Species and *Poncirus trifoliata*[a]

		mg/g (dry weight)		
seed parent × pollen parent	tentative code	peel	juice sac	edibility[b]
C. iyo × *P. trifoliata*	IyP267	1.586	1.685	
	IyP269	1.494	1.736	**
	IyP271	4.910	1.435	
	IyP272	0.909	1.235	*
	IyP277	2.487	3.794	*
	IyP279	2.097	0.862	*
	IyP280	2.952	2.957	
	IyP283	9.809	6.229	
	IyP289	3.225	6.228	
	IyP386	3.424	5.425	
	IyP387	9.005	5.299	*
	IyP389	5.874	3.934	*
	IyP804	1.345	0.338	
	IyP805-1	2.943	0.963	
C. unshiu "Miyasako" × *P. trifoliata*	MyP12-1	0.813	1.155	*
C. unshiu "Matsuki-wase" × *P. trifoliata*	MtWP296	3.539	3.200	
	MtWP298	1.638	0.279	
	MtWP400	4.359	2.420	
	MtWP402	6.613	2.945	
C. unshiu "Suzuki-wase" × *P. trifoliata*	SzWP262	3.364	0.243	
	SzWP264	2.025	2.811	*
	SzWP266-1	2.149	1.203	
C. unshiu "Miyagawa-wase" × *P. trifoliata*	MWP100	0.747	0.714	
	MWP242	1.132	0.673	
	MWP243	0.454	0.680	
	MWP244	6.425	1.034	
C. unshiu "Satou" × *P. trifoliata*	StP310	2.289	3.303	
C. unshiu "Sugiyama" × *P. trifoliata*	SgP131	10.735	7.480	
	SgP304	16.571	8.686	
	SgP306	2.187	2.900	
	SgP307	5.461	6.578	
C. junos × *P. trifoliata*	JP85	0.514	0.153	
	JP246	10.545	10.321	
	JP248	2.242	4.430	
C. kinokuni "Hira-kishiu" × *P. trifoliata*	HkP62	7.416	4.488	
	HkP114	6.528	0.319	*
	HkP115	6.475	5.217	
	HkP116	8.559	4.625	
	HkP119	8.380	5.753	
	HkP120	7.635	2.086	
	HkP300	7.000	3.051	
C. natsudaidai × *P. trifoliata*	NtP595	1.720	3.735	
C. nobilis "Kunenbo" × *P. trifoliata*	KnP42	7.575	1.971	
	KnP113	7.626	3.787	
C. tachibana × *P. trifoliata*	TP129	1.676	1.306	
	TP571	2.405	6.269	
C. hassaku × MtWP400	H (MtWP400)56	0.004	nd[c]	
	H (MtWP400)59	0.733	0.039	*
(*C. unshiu* × *C. sinensis*, Kiyomi) × IyP804	Ky (IyP804)3	0.002	0.024	
(*C. unshiu* × *C. sinensis*, Kiyomi) × StP310	Ky (StP310)3	0.006	0.106	
C. sinensis × (*C. paradisi* × *P. trifoliata*)	US119	nd	0.003	

[a] Specimens were collected on November 1997. [b] Edibility is indicated by the residual of smell and taste characteristic of *Poncirus* fruit: **, taste and smell disappear; *, slight residual taste and smell; blank, inedible. [c] nd, not detected at a detection limit (0.0001 mg/g).

hybrids IyP804 and StP310 have given rise to Ky(IyP804)3 and Ky(StP310)3. While auraptene content in the juice sac of Ky(IyP804)3 is only 0.024 mg/g, Ky(StP310)3 maintains a considerable amount of auraptene (0.106 mg/g) in its juice sac. We think that edible hybrids having both high auraptene content and low-acidity juice sac will originate from hybrid progenies of IyP269 crossed with *Citrus* species containing auraptene.

Citrus Fruit Products. The results of the analysis of auraptene content in citrus fruit products are shown in Table 4. Since grapefruit, Natsudaidai, and Yuzu are sources of auraptene, we assumed that the products made from those fruits contain auraptene. The process of making juice involves steps, such as the removal of pulp and peel oil, that cause a decrease in auraptene content. As a result of the analysis, auraptene was detected in two grapefruit juices. Its content in the juice is rather low (0.125 mg/100 g on the average), but the juice is the most familiar source of auraptene. Sweet oranges contain only a trace of auraptene in both the peel and the juice sac, and, indeed, orange juice products lack auraptene. Two brands of marmalade made from

Table 4. Auraptene Content in Citrus Fruit Products

item		mg/100 g (fresh product)
grapefruit juice	A	0.11
	B	0.14
orange juice		nd[a]
marmalade	A	0.38
	B	0.35
Ponzu		0.14
Yuzumiso		0.06

[a] nd, not detected at a detection limit (0.0001 mg/g).

sweet orange and Natsudaidai contained auraptene (0.35−0.38 mg/100 g). Though making marmalade involves a heating process, which causes the decomposition of auraptene and a decrease of its content, some amount of auraptene (0.37 mg/100 g on the average) remains in the marmalade. Ponzu and yuzumiso also contained auraptene (0.14 and 0.06 mg/100 g, respectively). Yuzu contains a small amount of auraptene in its juice sac (0.055 mg/g). Since ponzu contains peel oil, the auraptene in ponzu is probably derived from the peel.

Conclusion. In the present study, we have identified those citrus species, including hybrid progenies, that have high auraptene content. We also demonstrated the possibility of producing citrus enriched with auraptene. While a recommended intake of auraptene has not yet been established, it is clear that we do ingest auraptene from particular citrus fruits and their products. Knowing the auraptene contents in citrus fruits may assist in substantiating the relationship between cancer prevention and dietary intake of citrus fruits and their products.

ACKNOWLEDGMENT

We thank Dr. A. Murakami (Department of Biotechnological Science, Kinki University) for his generous supply of auraptene.

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Received for review May 24, 1999. Revised manuscript received January 7, 2000. Accepted February 18, 2000. This work was supported by the Program for the Promotion of Basic Research Activities for Innovative Biosciences from the Biooriented Technology Research Advancement Institution.

JF9905525