Amounts and variation in grapefruit juice of the main components causing grapefruit–drug interaction

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Abstract

A method for the determination of three furocoumarins containing two new chemicals (GF-I-1 and GF-I-4) in commercially available grapefruit juice and grapefruit itself was developed using high-performance liquid chromatography (HPLC). These components isolated from grapefruit juice have 5-geranyloxyfurocoumarin dimer structures showing extremely high affinities for a form of cytochrome P450 (CYP3A4). Considerable differences were observed on the contents among commercial brands and also batches. The contents were determined to be 321.4 ± 95.2 ng/ml GF-I-1, 5641.2 ± 1538.1 ng/ml GF-I-2 and 296.3 ± 84.9 ng/ml GF-I-4 in twenty-eight white grapefruit juices. These chemicals were not detected in beverages from orange, apple, grape and tangerine, except that trace amount of GF-I-2 and GF-I-4 were found in lemon juice. The average levels of these furocoumarins were lower in the juice from red grapefruit than a white one. The highest level of these components were found in the fruit meat. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Grapefruit juice; Cytochrome P450 inhibitors

1. Introduction

Grapefruit juice has been shown to elevate the pharmacological efficacy of orally administered drugs, such as felodipine [1], cyclosporin [2], terfenadine [3], midazolam [4] and lovastatin [5], and sometimes evoke side reactions through yielding conditions with the extraordinary high plasma concentration. Bioavailability of these drugs after oral administration are increased 1.5 to 15-fold by the ingestion of grapefruit juice. Data compiled on this drug interaction suggest several essential properties of drug influenced: All the drugs are lipophilic and require the extensive oxidative biotransformation prior to the excretion. In addition, human studies in vitro [6,7] and in vivo [8,9] indicate the major role of CYP3A4 on the oxidative metabolisms. These lines of evidence suggest the involvement of specific grapefruit juice component on the first pass metabolism of drugs which is mediated by intestinal CYP3A4.

Flavonoid components, particularly naringin, are contained abundantly in this juice, as the major bitter principle [10], and thus their inhibitory properties were examined. Naringenin, an aglycone generated

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by hydrolysis of naringin in intestine [11], inhibit felodipine metabolism in vitro [6,7]. However, naringin is shown to produce a negligible interaction with felodipine in vivo despite being given at a similar amount as that found in the grapefruit juice [12]. Consistent with the result, the inhibitory effect of ethyl acetate-extracts of grapefruit juice on microsomal CYP3A4 activity was not accounted for by the amounts of naringenin in the juice in our previous study [13]. To identify the component responsible for the inhibition of CYP3A4 activity, we examined the inhibitory effect of the extract by fractionation to isolate its inhibitory components. As the results, two new chemicals showing extremely high affinities for a form of cytochrome P450 (CYP3A4) were identified as 4-[[6-hydroxy-7-[[1-[(1-hydroxy-1-methyl)-ethyl]-4-methyl-6-(7-oxo-7*H*-furo[3,2-*g*][1]benzo-pyran-4-yl)-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]-oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one   (GF-I-1) and 4-[[6-hydroxy-7-[[4-methyl-1-(1-methylethenyl)-6-(7-oxo-7*H*-furo[3,2-*g*][1]benzopyran-4-yl)-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]oxy]-7*H*-furo[3,2-*g*][1]benzopyran-7-one (GF-I-4) to have 5-geranyloxyfurocoumarin dimer structures [14].

Grapefruits are natural products and, thus, the furocoumarin contents are expected to show considerable changes among the juices. In addition, wide variation are known on the consequence of grapefruit juice-induced drug interaction [8,9]. Grapefruit juices are daily consumed and often taken together with drugs. Therefore, differences in furocoumarin contents in commercial grapefruit juice and grapefruit itself have been investigated as a basis of prediction for grapefruit–drug interaction in the present study.

2. Experimental section

2.1. Apparatus

All mass spectra were obtained using a MStation 700 tandem type mass spectrometer (JOEL, Japan) equipped with an electrospray ionization source. An HPLC model HP1100 system (Hewlett-Packard, USA) was used. For ESI/MS analysis, the temperatures of the desolvating plate and orificel were set at 200°C and 80°C, respectively. The voltages of the ring lens and orificel were set at 90 V and 50 V,

respectively. ESI/MS was carried out using nitrogen to assist nebulization. Collisionally-induced dissociation (CID) was done with xenon at a collision energy of 1 kV. For a hydrogen–deuterium (H–D) exchange experiment [15], the sample was dissolved in the mobile phase containing deuterium oxide instead of water.

HPLC analysis was performed with a Chemcosorb 5-ODS-H (5 μm, 150×6.0 mm I.D., Chemco Scientific Co., Ltd., Osaka, Japan) equipped with a precolumn packed with Nucleosil 120-5C$_{18}$, (5 μm, 30×4.6 mm I.D., Chemco Scientific Co., Ltd., Osaka, Japan) using a Jasco Model PU-980 HPLC pump. Samples were introduced on the column via a Waters Model 712 Wisp autosampler. Metabolites were detected by the absorbance at 240 nm using a Jasco Model UV-970 variable wavelength UV–visible detector at room temperature. The mobile phase consisted of a multiple gradient of solvent A (water) and solvent B (methanol): 0 min, 40% (A); 30 min, 10%; 50 min, 5%; 60 min, 5%; 70 min, 40%, and set at the flow-rate of 1 ml/min.

2.2. Materials

Two new chemicals, GF-I-1 and GF-I-4 were separated from grapefruit juice as described in our previous study [14]. The mass spectra of GF-I-1 and GF-I-4 are shown in Fig. 1a and b. The protonated molecular ions produced at m/z 727 (GF-I-1) and m/z 709 (GF-I-4). These ions shifted at m/z 730 (GF-I-1) and m/z 711 (GF-I-4) after hydrogen–deuterium exchanges, confirming that GF-I-1 and GF-I-4 have two and one labile hydrogen in each molecule, respectively (Fig. 1c and d). Authentic GF-I-2 (bergamottin) and an internal standard, 8-decanyloxypsoralen, were synthesized by a method of A. McLillop et al. [16] and identified by NMR and mass spectrometry.

Grapefruit juice (total: Thirty-two samples) including two different types (white or red grapefruit) or ten different brands, and other juices were purchased from local commercial sources. The solvent, methanol, was HPLC grade from Nakalai (Kyoto, Japan). Water used for HPLC was filtered and passed through a Millipore Norganic cartridge before the mixing with HPLC-grade methanol.
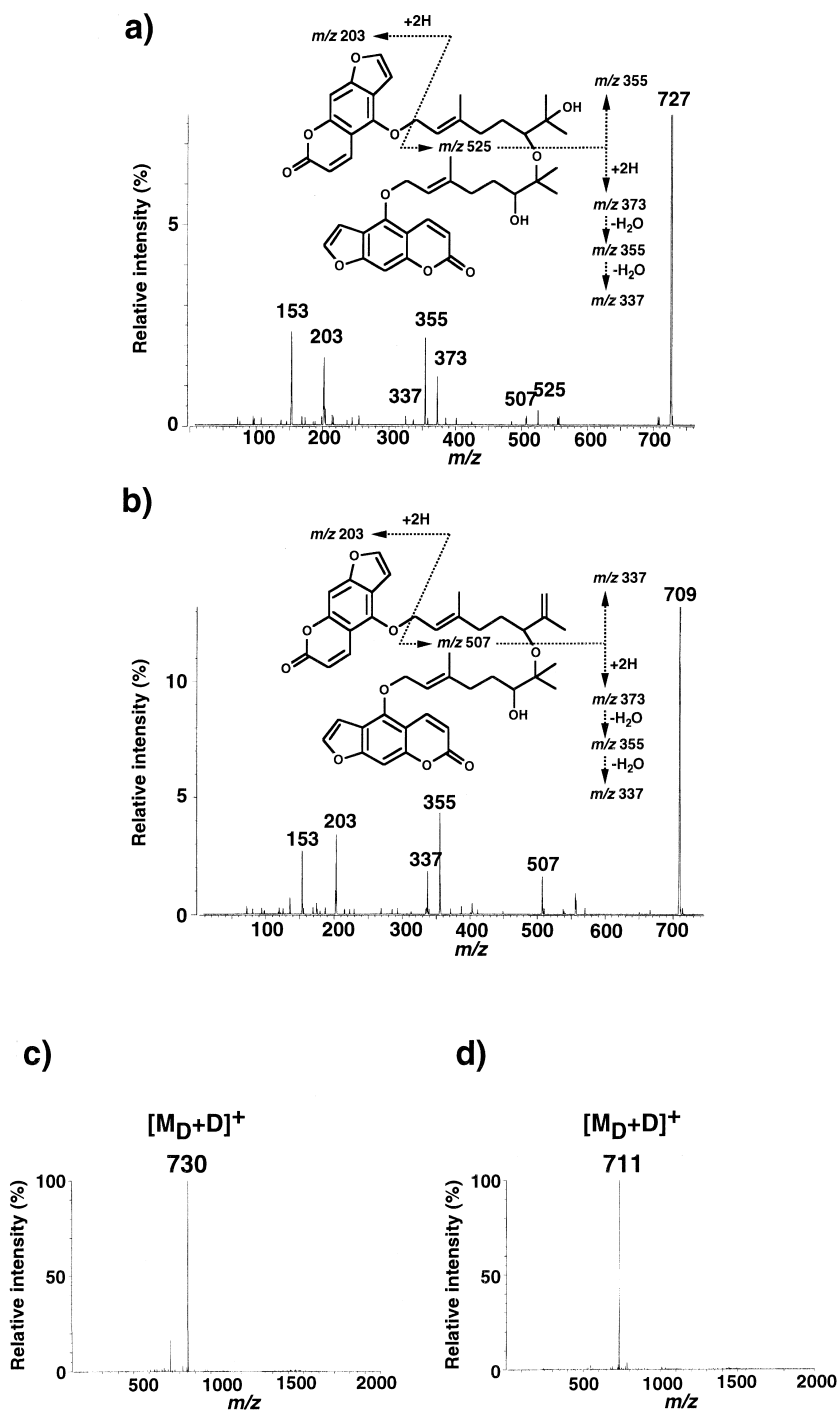
Fig. 1. Product ion spectra and mass spectra of furocoumarins. For sample preparation and analytical condition, see the Experimental. (A) Product ion spectrum of GF-I-1 produced by CID of the [M+H]$^+$ ion (m/z 727); (B) product ion spectrum of GF-I-4 produced by CID of the [M+H]$^+$ ion (m/z 709); (C) mass spectrum of GF-I-1 obtained with H–D exchange ([M$_D$+D]$^+$: m/z 730), and (D) mass spectrum of GF-I-4 obtained with H–D exchange ([M$_D$+D]$^+$: m/z 711).

2.3. Sample preparation

GF-I-1, GF-I-2 and GF-I-4 were diluted to make methanolic standard solutions (28.1–900.0 ng/50 µl for GF-I-1 and GF-I-4, and 281.3–9000.0 ng/50 µl for GF-I-2). These standard solutions (50 µl) after the addition of an internal standard, 8-decanyloxypsoralen (800 ng), were dissolved again in a mobile phase (final volume of 250 µl), and then an aliquot (90 µl) was injected to HPLC analyses after filtration. Grapefruit juice or other juices (1 ml) were extracted with 4 ml of ethyl acetate after the addition of the internal standard. For the determination of furocoumarins in grapefruit tissue, the fruit was divided into four parts, the peel, sac, fruit meat (flesh) and seed. Each part was homogenized by polytron after the addition of 3 to 10-fold amounts of water and a portion (1 ml) of the resultant homogenate was extracted with 4 ml of ethyl acetate after being mixed with the internal standard. The residue, after evaporation, was reconstituted in 250 µl of mobile phase and an aliquot of 90 µl was injected into the HPLC.

2.4. Calibration and recovery

Calibration curves were generated using six different concentrations of standard solutions of GF-I-1, GF-I-2 and GF-I-4 from least-squares regressions of peak area of these analytes versus internal standard. The intra-day precision and accuracy of the quantification were evaluated by replicate analyses ($n=4$) of the standards. Precision was based on the calculation of the coefficient of variation (C.V.). Accuracy was based on the calculation of the relative error (R.E.) of the found amount compared to a theoretical one. For a recovery study, grapefruit juice were divided into two portions. Half of the juice was spiked with 40, 180 or 360 ng of GF-I-1 and GF-I-4, and 1000, 2000 or 4000 ng of GF-I-2 and then processed as described above. The peak areas corresponding to spiked GF-I-1, GF-I-2 and GF-I-4 were calculated from the differences of peak areas between spiked and non-spiked juices. The recovery was determined by comparing the peak area of spiked GF-I-1, GF-I-2 and GF-I-4 with the standard solution.

3. Results

3.1. Calibration and recovery

An adequate separation of all analytes were achieved as shown in Fig. 2. Linearity of GF-I-1, GF-I-2 and GF-I-4 was evaluated over the range 10.1–324.0 ng per injection for GF-I-1 and GF-I-4, and 101.3–3240.0 ng per injection for GF-I-2. The calibration curves of GF-I-1, GF-I-2 and GF-I-4 were $y = 0.00108x + 0.00368$ ($r = 0.999$), $y = 0.00115x + 0.0444$ ($r = 0.999$) and $y = 0.00100x + 0.00224$ ($r = 0.999$), respectively. The intra-day variability of GF-I-1, GF-I-2 and GF-I-4 are summarized in Table 1. The intra-day C.V. values were less than 5.4% and the intra-day accuracies were between -5.2 and 2.7%, within the amount range of the calibration curves for these analytes. The limits of quantification for GF-I-1, GF-I-2 and GF-I-4 were set to 10.1 ng, 101.3 ng and 10.1 ng per injection, respectively, which are the lowest amounts that could be measured with acceptable C.V. ($<20\%$) and accuracy ($<20\%$). The recoveries from grapefruit juice spiked were between 94.2 and 104.7% for GF-I-1, 92.0 and 108.4% for GF-I-2 and 83.1 and 97.0% for GF-I-4, respectively.

3.2. Concentration of GF-I-1, GF-I-2 and GF-I-4 in juices

To evaluate the concentration of GF-I-1, GF-I-2 and GF-I-4 among juices of different types (white or red grapefruit), brands and batches, commercially available, thirty-two different grapefruit juices were used in the analysis. Considerable differences were observed on the amounts among brands and also batches. White grapefruit contained 321.4±95.2 ng/ml of GF-I-1, 5641.2±1538.1 ng/ml of GF-I-2 and 296.3±84.9 ng/ml of GF-I-4 (Table 2). The average contents of furocoumarins in pink grapefruit juices, which were made from rubyred grapefruit, were determined as 184.8±96.7 ng/ml GF-I-1, 3740.0±1443.3 ng/ml GF-I-2 and 209.1±103.5 ng/ml GF-I-4. To know whether these furocoumarins are contained selectively in grapefruit juices, other fruit juices were also analyzed. As shown in Fig. 3, GF-I-1, GF-I-2 and GF-I-4 were not detected in
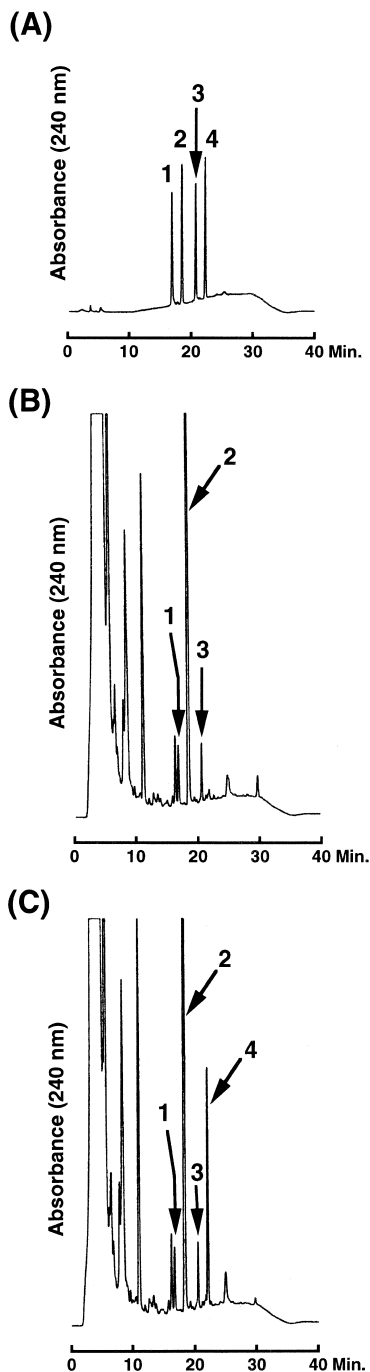
(A)

(B)

(C)

Fig. 2. HPLC separation of furocoumarins. For sample preparation and analytical condition, see the Experimental. Peaks: 1=GF-I-1, 2=GF-I-2, 3=GF-I-4, 4=internal standard. (A) Standard furocoumarins, (B) grapefruit juice, and (C) grapefruit juice spiked with internal standard.

Table 1
Intra-day precision and accuracy for analyses of GF-I-1, GF-I-2 and GF-I-4 ($n=4$)

Compounds	Amounts (ng/injection)	C.V.[a] (%)	Accuracy[b] (%)
GF-I-1	10.1	5.4	−5.1
	20.3	1.1	−1.8
	40.5	3.3	−0.4
	81.0	2.5	1.0
	162.0	1.0	0.3
	324.0	0.3	−0.1
GF-I-2	101.3	1.0	−4.9
	202.5	0.4	0.8
	405.0	2.4	2.3
	810.0	1.7	1.0
	1620.0	0.4	−1.3
	3240.0	1.7	0.2
GF-I-4	10.1	2.1	−5.2
	20.3	2.2	−4.5
	40.5	5.3	−0.4
	81.0	1.3	1.0
	162.0	2.0	0.8
	324.0	1.4	0.1

[a] C.V.=coefficient of variation.
[b] Accuracy=relative error.

juices from orange, apple, grape and tangerine, except that trivial amount of GF-I-2 and GF-I-4 were detected in lemon juice (Fig. 3).

3.3. Localization of GF-I-1, GF-I-2 and GF-I-4 in grapefruit

To assess tissue localization of furocoumarins in grapefruit, the fruit was divided into four parts, the peel, sac, fruit meat (flesh) and seed. Results on the qualification of GF-I-1, GF-I-2 and GF-I-4 in white grapefruit are shown in Fig. 4. These three furocoumarins were found to be contained in the flesh portion, followed by the sac, peel and seed in a decreasing order, except that GF-I-2 in the peel was higher than in the sac. Amounts of GF-I-1, GF-I-2 and GF-I-4 in white grapefruit were calculated as 207.7±55.0 μg, 4678.4±1276.0 μg and 167.7±44.8 μg per a grapefruit, respectively. Contents of these chemicals in rubyred grapefruit were less, but consistent with the results of commercial grapefruit juices.

Table 2
Concentration of furocoumarins in commercially available grapefruit juices

Commercial product	n	White/Pink	GF-I-1 (ng/ml)	GF-I-2 (ng/ml)	GF-I-4 (ng/ml)
Tropicana	8	White	363.3±34.1	5455.1±492.2	292.4±41.5
Dole	5	White	348.9±15.8	5105.8±98.5	288.8±19.5
Sunkist	5	White	346.4±24.7	5868.1±278.3	299.6±29.5
Welch	3	White	352.0±90.5	6991.7±1961.8	351.4±131.4
Kagome	3	White	202.8±173.6	4416.3±2812.6	250.4±167.7
Sunpokka	1	White	392.0	7400.3	374.6
Berri	1	White	312.5	9985.2	436.4
Takano	1	White	210.0	4136.6	162.7
Liby	1	White	54.3	4922.1	164.4
Total	28	White	321.4±95.2	5641.2±1538.1	296.3±84.9
Welch	1	Pink	138.4	3888.5	272.2
Sunpokka	1	Pink	296.0	5452.1	308.5
Texun	1	Pink	120.1	3694.2	178.2
Liby	1	Pink	N.D.[a]	1925.2	77.6
Total	4	Pink	184.8±96.7[b]	3740.0±1443.3	209.1±103.5

[a] N.D. = Not detected.
[b] This result is reported as the mean±SD of three samples.

4. Discussion

Coadministration of grapefruit juice with drugs, metabolized mainly by CYP3A4, has been reported to result in substantial increases in their oral bioavailabilities [1–5]. This phenomenon occurred through the inhibition of CYP3A4. In our previous study [14], GF-I-1 and GF-I-4 were shown to inhibit microsomal testosterone 6β-hydroxylation in human livers. The present study indicates that GF-I-1 and GF-I-4 are specifically contained in grapefruit juice, but not in other fruit juices of daily consumption.

As shown in Table 2, GF-I-1, GF-I-2 and GF-I-4 were contained in all thirty-two different samples of grapefruit juices. The contents of these three chemicals differed considerably among samples, particularly on GF-I-1. The average levels of GF-I-1, GF-I-2 and GF-I-4 were higher in juices from white grapefruit than red grapefruit, although some red grapefruit juice contained these furocoumarins at levels similar to the average level of white grapefruit.

GF-I-2, a presumed precursor of GF-I-1 and GF-I-4, is known to be an ingredient of grapefruit essential oil and bergamot oil [17]. In the present study, grapefruit juice is shown to contain 17-fold higher amount of GF-I-2 than that of GF-I-1. GF-I-2

inhibited CYP3A4-dependent oxidation in human liver microsomes, but the effect is at least one order of magnitude weaker than GF-I-1 or GF-I-4 (unpublished data). Clear inhibition of CYP3A4 activity was observed in the fraction containing GF-I-1 and GF-I-4, whereas only a marginal inhibitory effect was observed in the fraction containing GF-I-2 in our previous paper [14]. These results suggest the major contributors are GF-I-1 and GF-I-4, although all furocoumarin components would be active ingredients responsible for grapefruit juice interaction.

Consistent with selective occurrence of drug interaction with grapefruit juice [18,19], these furocoumarins were not detected in other beverages from fruits such as orange, apple, grape and tangerine, except that trace amounts of GF-I-2 and GF-I-4 were found in lemon juice. These results also support the idea that GF-I-1 and GF-I-4 would be causative components, although a definitive conclusion should not be made at this moment.

To estimate the localization of these furocoumarins, grapefruit was divided into four parts, the peel, sac, fruit meat (flesh) and seed. Results of the analysis by HPLC showed that the three components were found in the fruit at the highest level. Concentration of these furocoumarins in the fruit of white grapefruit were determined to be
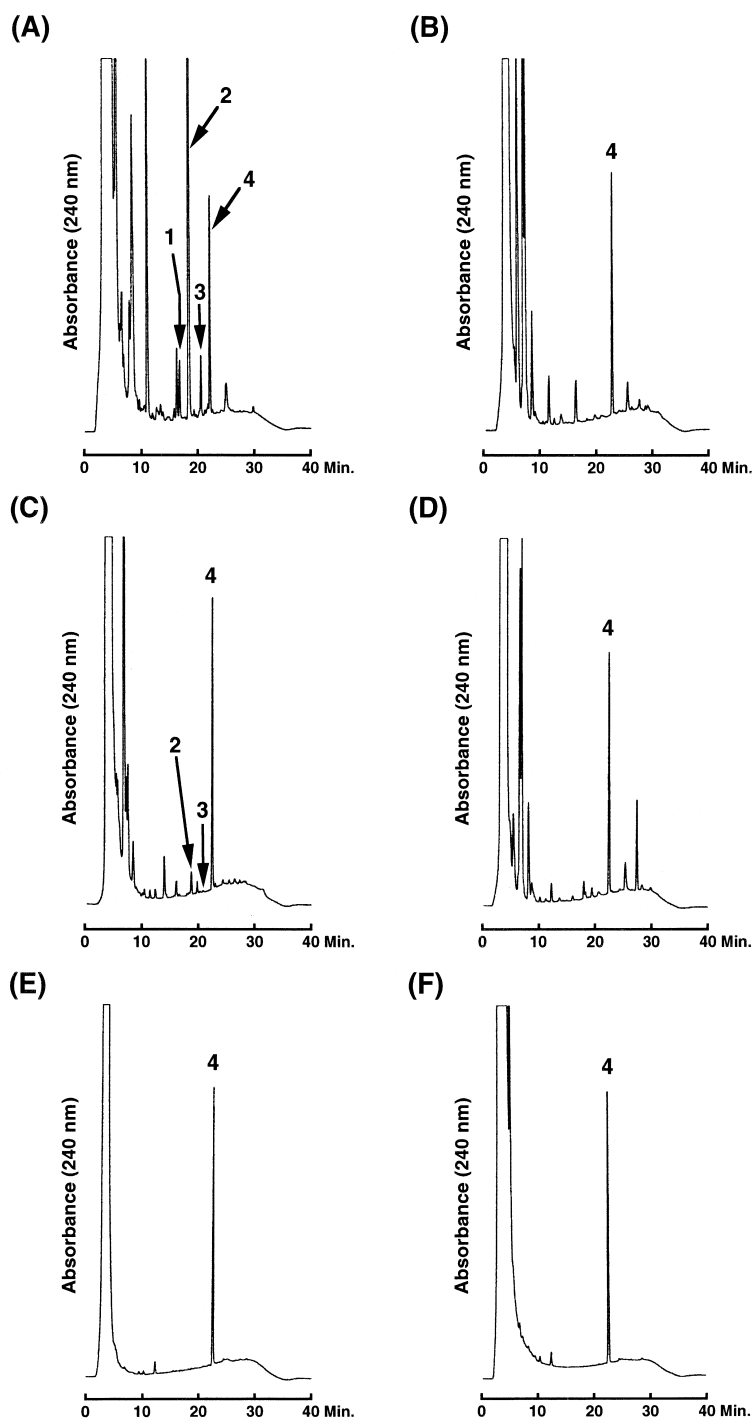
Fig. 3. HPLC separation of furocoumarins in citrus juices. For sample preparation and analytical condition, see the Experimental. Peaks: 1=GF-I-1, 2=GF-I-2, 3=GF-I-4, 4=internal standard. (A) Grapefruit, (B) orange, (C) lemon, (D) tangerine, (E) apple and (F) grape.
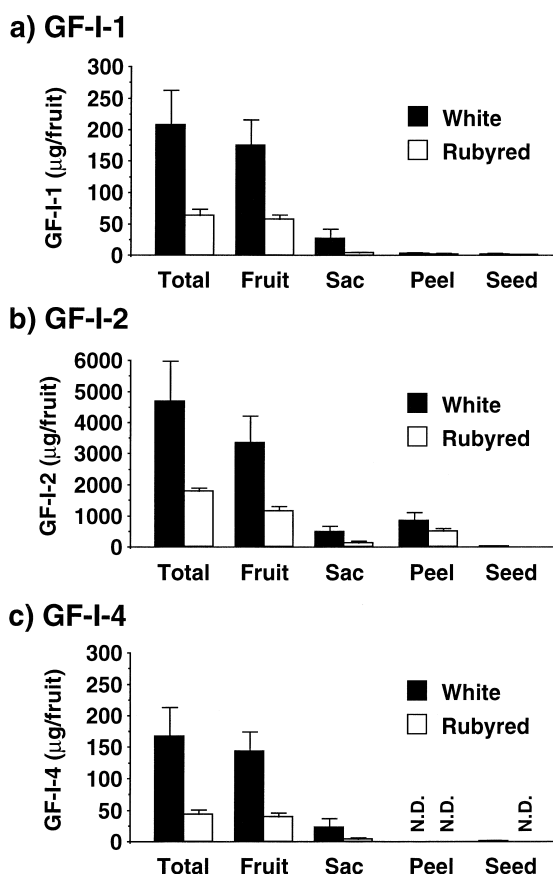
a) GF-I-1

b) GF-I-2

c) GF-I-4

Fig. 4. Localization of furocoumarins in grapefruit tissue. For sample preparation and analytical condition, see the Experimental. (A) GF-I-1, (B) GF-I-2, (C) GF-I-4.The results are represented as the mean±SD of three grapefruits.

599 ng/g, 11 418 ng/g and 489 ng/g, for GF-I-1, GF-I-2 and GF-I-4, respectively. Consistent with the results of commercially available grapefruit juice, contents of these furocoumarins were less in rubyred grapefruit than in white ones. It might be calculated that ingestion of 100 g of the fruit flesh, corresponding to about a third of the whole fruit, would be equivalent to 200 ml of regular strength grapefruit juice. The amount is known to increase plasma concentration of felodipine [12].

The C-1 and C-6 positions in two geranyl moieties of GF-I-1 and GF-I-4 are chiral centres. In our preliminary experiment using a reversed-phase HPLC equipped with a chiral column, predominance of a stereoisomer was detected by analysis of 6′,7′-

dihydrobergamottin isolated from grapefruit juice, although the absolute configuration remains undefined. The enantiomer excess was 95% as compared with 50% in an synthetic standard of this chemical (data not shown). The determination of the absolute configuration and the chemical synthesis for GF-I-1 and GF-I-4 are in progress.

5. Definition list

HPLC	high-performance liquid chromatography
CYP and P450	cytochrome P450
LC/MS/MS	liquid chromatography/tandem mass spectrometry
NMR	nuclear magnetic resonance
CID	collisionally induced dissociation
H–D	hydrogen–deuterium
GF-I-1	4-[[6-hydroxy-7-[[1-[(1-hydroxy-1-methyl)ethyl]-4-methyl-6-(7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl)-4-hexenyl]oxy-3,7-dimethyl-2-octenyl]oxy]-7H-furo[3,2-g][1]benzopyran-7-one
GF-I-4	4-[[6-hydroxy-7-[[4-methyl-1-(1-methylethenyl)-6-(7-oxo-7H-furo[3,2-g][1]benzopyran-4-yl)-4-hexenyl]oxy]-3,7-dimethyl-2-octenyl]oxy]-7H-furo[3,2-g][1]benzopyran-7-one

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K. Fukuda et al. / J. Chromatogr. B 741 (2000) 195–203

203

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