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Cucurbitacin C—Bitter Principle in Cucumber Plants

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Abstract

Cucumber plants contain a bitter substance, cucurbitacin C. The compound was isolated from cucumber leaves using preparatory HPLC (high performance liquid chromatography) to investigate the relationship between its content and bitterness of the plant parts. An analytical method for cucurbitacin C using HPLC was also established. A Japanese popular cultivar, 'Sharp 1' contained the compound in the leaves but not in the fruits, while a unique cultivar with white skin 'Shinsyo Hakuhi' contained it both in leaves and fruits. The stem end of 'Shinsyo Hakuhi' fruit contained higher amounts of it than other fruit parts. Cucurbitacin C is a strongly bitter component and its threshold level was less than 0.1 mg/L. The bitter sensation felt when biting the plant parts could be interpreted as corresponding to the content of cucurbitacin C.

Discipline: Horticulture
Additional key words: analysis, *Cucumis sativus* L., HPLC, isolation

Introduction

Plants belonging to the Cucurbitaceae family contain bitter triterpenes called cucurbitacins[2]. Cucumber plants (*Cucumis sativus* L.) are known to contain only one form of cucurbitacins, cucurbitacin C[13]. The bitterness of cucumber fruit is thought to be caused by cucurbitacin C, and the bitterness of the fruit of a local cucumber cultivar 'Kagafutokyuri' was studied by Kano et al.[6–10]. In spite of their magnificent research they did not analyze the compound itself; probably they could not obtain it as the standard for the analysis.

These days many people are interested in the beneficial effects of food on health, and cucurbitacins have been studied for their anti-tumor activities[11]. It is expected that cucumber fruits have such effects[5] since they have been reported to contain cucurbitacin C[13]. Before discussing the effects of eating cucumber fruits, cucurbitacins in the fruits must be quantified.

No report has been published on the quantification of the contents of cucurbitacins in the fruits or the other parts of cucumber cultivars, or on interpreting qualitatively the bitterness of cucumber fruit caused by the compound. The analysis of cucurbitacin C in cucumber plants was done to solve these problems. In this paper, we report (1) the isolation of cucurbitacin C to obtain the standard for the analysis, (2) the analytical methods for cucurbitacin C using high performance liquid chromatography (HPLC), and (3) the results of the analysis comparing the difference among the cucumber cultivars. We also discuss the relationships between the contents of cucurbitacin C and the bitterness on eating the fruits.

Materials and methods

1. Isolation of cucurbitacin C

Young leaves of cucumber plants were harvested and stored at -20ºC until the extraction. Frozen leaves were crushed in petroleum ether and the residue was obtained after vacuum filtration. The residue was extracted with methanol and the methanol extract was concentrated under reduced pressure, then it was dissolved in water and successively extracted with petroleum ether and dichloromethane. The dichloromethane extract was evaporated and suspended in a small amount of methanol, according to Peters et al.[12]. Cucurbitacin C was further purified using HPLC. The HPLC system used for the preparation consisted of a pump, injector and photodiode array detector. Five mL of the methanol suspension was injected into

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the HPLC system, using methanol : water (55 : 45) solvent at 4 mL/min. The column used was CAPCELLPAK C18 UG80 (250 mm × 20 mm i.d., Shiseido, Japan). The peak of the cucurbitacin was identified based on the spectra obtained with the photodiode array detector as referred to by Enslin et al.[3]. The collected fraction was freeze-dried and the white powder was obtained.

The compound isolated showed just one peak as a result of the analysis using HPLC. The EI mass spectrum showed characteristic fragments of m/z 470, 482 and 500 for cucurbitacin C reported by Rice et al.[14]. The ultraviolet absorption spectrum and the molar extinction coefficient of the compound agreed with the results reported by Enslin et al.[3]. It was identified as cucurbitacin C and used as the standard for the following analysis.

2. Analytical conditions for cucurbitacin C

The contents of cucurbitacin C in cucumber plants were determined using gradient HPLC. The HPLC system used was Agilent 1100 series (with a binary pump and UV-VIS detector) in combination with a 150 × 4.6 mm i.d. ZORBAX Eclipse XDB-C8 column (particle size: 5 μm, Agilent, USA). Gradient elution was performed using water (A) and water-acetonitril (20 : 80, v : v) (B) as mobile phases, delivered at a flow rate of 1.0 mL/min as follows: initially 10% of mobile phase B for 2 min, on a gradient starting with 10% B to reach 100% B at 15 min. The column temperature was set at 40ºC, and the UV signals were monitored at 230 nm.

3. Sample preparations

The cucumber cultivars were cultivated in a greenhouse at the National Institute of Vegetable and Tea Science in Tsu, Mie Prefecture, Japan. The samples for the analysis were collected in the spring and summer of 2004 and 2005. Fresh leaves and buds were crushed and extracted in methanol. For the analysis of the fruit, the juice squeezed by a garlic press was used as the sample extract. Five μL of the sample, filtered with a membrane filter (pore size: 0.45 μm) in advance, was directly injected into the HPLC system.

Results and discussion

The method for the rapid HPLC analysis of cucurbitacin C in cucumber cotyledons has already been published by Gorski et al.[4]. Although their method is very simple, the separation was not good enough for our investigations. A gradient system was introduced for our analysis to improve the separations. Satisfactory separation was attained using a C8 column instead of C18 columns. The chromatogram for the analysis of cucumber leaves is shown in Fig. 1.

First we investigated the difference in contents of the compound among the parts of the plant. It is supposed that showing the average of the analytical results is not suitable in this case, because the contents varied extensively even in the same plant parts collected on the same day. The most representative results are shown in Table 1. The cultivar described here was the most popular one in Japan, 'Sharp 1'. The youngest leaves at the top of the stem (the tip) tended to contain the highest amount of cucurbitacin C. We have never detected the compound in the fruit of this cultivar during the three years of our experimental period (see Table 2); however, young buds of the female flower contained it although the contents were not high (Table 1). Organoleptically, the tip of the stem and young leaves were bitter, the buds were slightly bitter and the fruits were non-bitter. These sensations of bitterness correspond to the cucurbitacin C contents shown in Table 1, supporting the hypothesis that the bitterness of the cucumber plants was caused by cucurbitacin C.

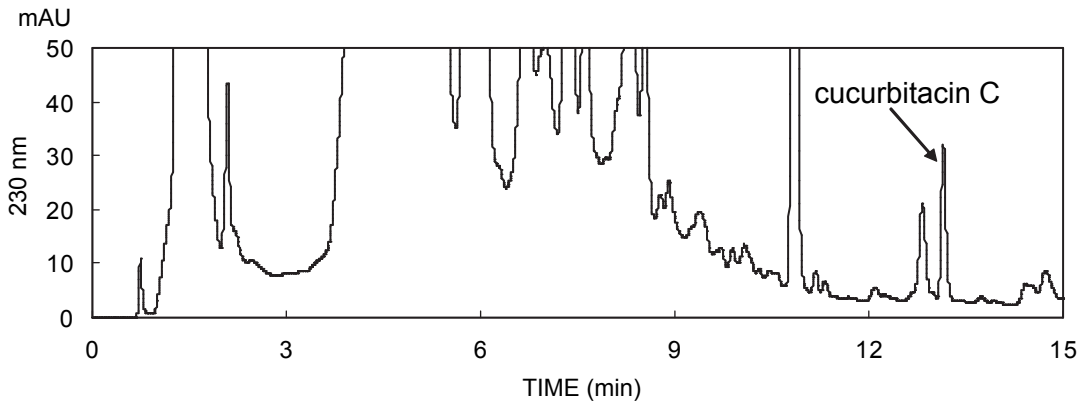

Fig. 1. Chromatogram of the methanol extract from the young leaf of a popular cultivar 'Sharp 1'

Table 1. Comparison of the cucurbitacin C contents among different parts of one cucumber plant

	Cucurbitacin C (mg/kg FW)
Tip of the stem	29.9
3rd leaf	24.0
Female flowers*	0.4
Fruit	ND

*: Four buds were used for the analysis. One example of the analysis of the main stem of the cultivar 'Sharp 1' is shown.

Table 2. Comparison of the cucurbitacin C contents and bitterness among cultivars

Cultivar	Cucurbitacin C contents (mg/kg FW)		Bitterness	
	Tip of the stem	Fruit	Leaves	Fruit
Sharp 1	34.0±10.7	ND	b	n
Zenkoku Suyo Kyuri	27.6±11.0	ND	b	n
Marianna RZ	ND	ND	n	n
Shinsyo Hakuhi	13.7± 4.1	2.0±3.5*	b	b

*: Juice prepared from the stem end of the fruit (mg/L). Values are the mean ± standard deviation (n = 4). ND: Not detected, b: Bitter, n: Non-bitter.

The contents of cucurbitacin C and organoleptic bitterness were compared among the cucumber cultivars (Table 2). The tips of the stems were used for the analysis of cucurbitacin C, since the variation of the contents among the tips was relatively smaller than that among the leaves. Cucurbitacin C in the fruits was investigated by targeting the juice squeezed from the stem end (opposite the blossom end, see Fig. 2) of the fruit, since this part is said to be the bitterest, and the bitterness of the fruit was tested by biting this part of the fruit.

Cucurbitacin C was not detected either in the leaves or fruits of 'Marianna RZ', a genetically cucurbitacin-less cultivar belonging to the English greenhouse type variety, which corresponds to the non-bitter taste of these plant parts. The leaves of the other cultivars were bitter and they contained the compound in the tips of the stems. Popular cultivars in Japan ('Sharp 1' and 'Zenkoku Suyo Kyuri') did not show bitterness in the fruits and they contained no cucurbitacin C in the fruits, whereas the fruits of 'Shinsyo Hakuhi', which is a specialized cultivar with a unique white skin color, often showed strong bitterness and cucurbitacin C was occasionally detected in the stem end of the fruits. Although 'Shinsyo Hakuhi' was the only cultivar in our experiments that contained cucurbitacin C in the fruit, this cultivar did not always contain the highest amount of it in the leaves. The compound was detected in the leaves and buds of the female flowers (Table 1) but not in the fruits of 'Sharp 1'. It is supposed that the cultivars except for 'Marianna RZ' have the potential to produce cucurbitacin C, and there may be a difference in the controlling mechanisms to accumulate it in the fruit between 'Shinsyo Hakuhi' and Japanese popular cultivars.

It is commonly believed that the stem end of the cucumber fruits is bitterer than the middle or blossom end, so it is expected that cucurbitacin C is highly distributed in the stem end. The distribution of the cucurbitacin in one fruit was determined. The cultivar used was the bitterest one, 'Shinsyo Hakuhi'. Bitterness was often faint or very weak in the middle parts or blossom end of the fruit even in this cultivar. The cucurbitacin was not detected in these parts of such fruits. A strongly bitter fruit was selected for this experiment following the results of biting the middle part of the fruits. The concentrations determined from the juice of the slices are shown in Fig. 2. The concentrations were higher at the parts near the stem end. The concentration in the middle parts or blossom end was about 0.1 mg/L, which was almost the limit of quantitative analysis. It is concluded that the stem end is richer in cucurbitacin C and is bitterer than the other parts of the fruit.

Some literature reported the possibility of the antitumor effects of cucurbitacin C by eating cucumber fruits[5]. Our results show that one fruit (about 100 g FW) contains a very small amount (much less than 1 mg) of cucurbitacin C, even when selecting a strongly bitter fruit. Although some cucurbitacins have been reported to show cytotoxicity against cancer cell lines, there has been no report of beneficial effects on health using cucurbitacin C itself[1]. It is suggested that the beneficial effects of cucurbitacins in cucumber fruits be discussed much more carefully.

An aqueous solution of 0.1 mg/L of cucurbitacin C was still bitter, and it is suggested that the threshold level of the bitterness is less than 0.1 mg/L. The threshold

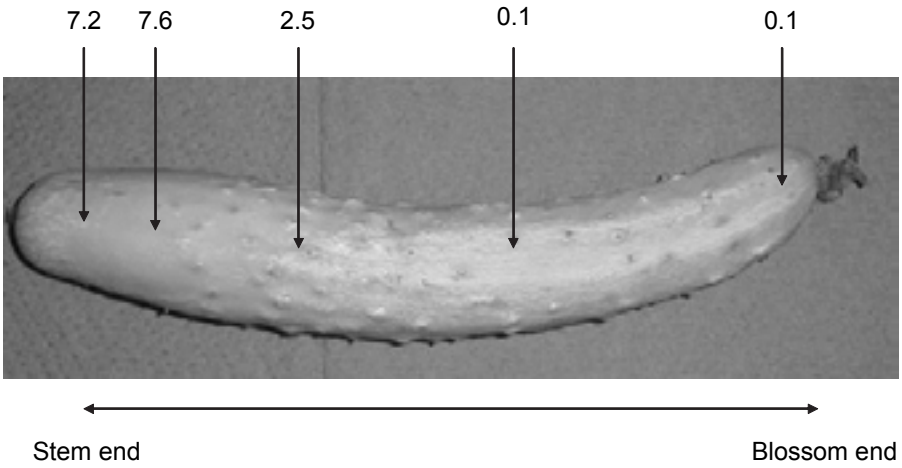

Fig. 2. The concentrations of cucurbitacin C in the juice prepared from different parts of one fruit of 'Shinsyo Hakuhi'
Values above each arrow indicate cucurbitacin C concentrations (mg/L) in the juice from different parts of the fruit.

level of caffeine, a typical bitter substance, is 150 mg/L. Cucurbitacin C is more than 1,000 times bitterer than caffeine. Kano et al.[6–10] estimated the existence of cucurbitacin C in a cucumber cultivar 'Kagafutokyuri' by biting the fruits and we suppose their biting method was effective for their purpose to detect the compound, because we have already declared the existence of cucurbitacin C in the bitter fruits of this cultivar (data not shown), and the HPLC method shown here is not more sensitive than the organoleptic assessment.

In conclusion, the bitterness of the cucumber plants could be interpreted as corresponding to the cucurbitacin C content. The popular Japanese cultivars have been selected to repress the production of the compound in the fruit, whereas in some local cultivars including 'Kagafutokyuri' the selection is still incomplete and bitter fruits are harvested occasionally. The methods described here could be useful tools to improve the quality of these local cultivars.

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