



Cucurbitacins in plant food

Jørn Gry, Inge Søborg and Hans Christer Andersson

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The Nordic Committee of Senior Officials for Food Issues is concerned with basic Food Policy issues relating to food and nutrition, food toxicology and food microbiology, risk evaluation, food control and food legislation. The co-operation aims at protection of the health of the consumer, common utilisation of professional and administrative resources and at Nordic and international developments in this field.

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Preface

The Nordic Committee of Senior Officials for Food Issues is an advisory body of the Nordic Council of Ministers which co-ordinates Nordic work in the field of food and nutrition. The Committee has given the Nordic Working Group on Food Toxicology and Risk Evaluation (NNT) the responsibility to promote co-operation and co-ordination among Nordic countries in matters relating to food toxicology and risk assessment.

Assessment of health risks connected with exposure to naturally occurring toxicants in foodstuffs has become an important area for NNT in the recent years. A series of Nordic reports based on the work performed by the Nordic project group on inherent natural toxicants in food plants and mushrooms has been published:

- Gry, J. and Pilegaard, K. (1991) Hydrazines in the Cultivated Mushroom (*Agaricus bisporus*). Vår Föda 43;Supplement 1.
- Uggla, A. and Busk, L. (1992) Ethyl carbamate (urethane) in alcoholic beverages and foodstuffs – A Nordic View. Nordiske Seminar- og Arbejdsrapporter 1992:570.
- Størmer, F.C., Reistad, R. and Alexander, J. (1993) Adverse health effects of glycyrrhizic acid in licorice. A risk assessment. Nordiske Seminar- og Arbejdsrapporter 1993:526.
- Andersson, C., Slanina, P. And Koponen, A. (1995) Hydrazones in the false morel. TemaNord 1995:561.
- Søborg, I., Andersson, C. and Gry, J. (1996) Furocoumarins in Plant Food – exposure, biological properties, risk assessment and recommendations. TemaNord 1996:600.
- Gry, J. and Andersson, H.C. (1998) Nordic seminar on phenylhydrazines in the Cultivated Mushroom (*Agaricus bisporus*). TemaNord 1998:539.
- Andersson, H.C. (1999) Glycoalkaloids in tomatoes, eggplants, pepper and two Solanum species growing wild in the Nordic countries. TemaNord 1999:599.
- Andersson, H.C. (2003) Calystegine alkaloids in Solanaceous plants. TemaNord: 2002:513.
- Andersson, H.C., Wennström, P. and Gry, J. (2003) Nicotine alkaloids in Solanaceous food plants. TemaNord 2003:531.
- Andersson, H.C. and Gry, J. (2004) Phenylhydrazines in the Cultivated Mushroom (*Agaricus bisporus*). TemaNord 2004:558, 123 pp.

Poisonings caused by cucurbitaceous vegetables seem to be linked to intake of immensely bitter vegetables. The bitter and toxic compounds in these vegetables are cucurbitacins, which are well known in wild varieties of these food plants and their related species. The cultivated forms, on the other hand, have during the time in cultivation been selected for being free of the bitter and toxic compounds.

This review summarises the information available on cucurbitacins in food plants of the family *Cucurbitaceae*, with the aim to lay down background information required to evaluate the potential risk of being intoxicated by cucurbitacins as a part of the safety assessment of cucurbita-

ceous food plants, and especially in relation to genetically modified Cucurbitaceous plants.

Information on cucurbitacins was collected from literature identified by searching Food Science Technology Abstracts, CAB-web, Toxline, Biosis, and Medline up to Dec 2004, using the search term cucurbitacin*. Additional references were identified from the reference lists of identified literature. However, a selection of the information has taken place in order to cover primarily plant foods consumed in the Nordic countries.

The *Project Group* consisted of the following members:

- Jørn Gry (co-ordinator)
Danish Institute for Food and Veterinary Research, Denmark
- Christer Andersson
National Food Administration, Sweden
- Jan Alexander
National Institute of Public Health, Norway
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The present report has been prepared by Jørn Gry¹, Inge Søborg¹, and Christer Andersson² after thorough discussions in the project group, and finally adopted by NNT.

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1. Summaries

1.1 English Summary

The report aims to give a comprehensive review on the occurrence of cucurbitacins in food plants of the family *Cucurbitaceae* and to highlight the potential toxicity of cucurbitacin-containing food items from cucurbitaceous plants.

Cucurbitacins are tetracyclic triterpenes with a cucurbitane skeleton. They differ from most other tetracyclic triterpenes by being highly unsaturated and containing numerous keto-, hydroxy- and acetoxy-groups. The cucurbitacins usually occur as β -2-monoglycosides, the sugar moiety being D-glucose or L-rhamnose. It is still discussed whether the cucurbitacin aglycones are present as such in the intact plant or whether the aglycones are found simply due to an extremely fast acting β -glycosidase turning active when cells are destroyed in preparation for the chemical analysis.

Cucurbitacins are highly toxic compounds which until now have been shown to occur in about 100 of the 900 species of *Cucurbitaceae*. The highest concentrations are normally found in roots, but it is never the roots that are consumed as food. It is usually the fruits. However, some roots have been used and some are still used in traditional medicines. No food plants from other plant families have ever been reported to contain cucurbitacins.

Fruits of the cultivated *Cucurbitaceae* – cucumber, squash and other pumpkins, calabash, melon, and watermelon – have all been cultured to become “free of cucurbitacins”. They are all assumed to contain a suppressor-gene or a mutation responsible for the absence of cucurbitacins, but back mutations occur randomly and may lead to plants with toxic and bitter fruits. Back-mutated watermelons and squash have been reported to produce between 930 and 3100 mg cucurbitacin E per kg fresh fruit. The offspring of such plants may also produce cucurbitacins. Chayote fruit and snake gourds are cucurbitaceous plants cultivated and eaten in other parts of the world that never have been found with bitter fruits. However, seedlings of both these plants are known to contain cucurbitacins, so the plants do have the genetic constitution to produce these compounds..

In the Nordic societies the only dietary exposure to cucurbitacins will be via fruits of back-mutated cucurbitaceous food plants. Cucurbitaceous fruits are normally sweet and free of these compounds. The cucurbitacins are extremely bitter – cucurbitacin E glycoside (the least bitter of the tested compounds) may be recognised at 2 ppm (2 mg/kg) when mixed with squash pulp. If the anticipated exposure to cucurbitacins arises from

one mouthful (around 20 g) of squash or watermelon containing between 930 and 3100 mg/kg, this corresponds to an exposure of 18–60 mg cucurbitacins in a person weighing 70 kg. This exposure will correspond to 0.3–0.9 mg/kg body weight. Repeated or chronic exposure to cucurbitacin is not expected due to their accidental occurrence in food plants and their extremely bitter and disagreeable taste.

There is no information available on absorption, metabolism and excretion of cucurbitacins or cucurbitacin glycosides. The toxic effects recorded in animals and man, however, indicate that these compounds are absorbed at least to some extent.

Exposures of experimental animals to cucurbitacins may lead to acute toxic effects. Although exposure via the oral route gives rise to less toxic effects than exposure via the subcutaneous, intraperitoneal and intravenous routes, the oral LD₅₀ in mice is as low as around 5 mg/kg body weight for some of the cucurbitacins (cucurbitacin D and I). Cucurbitacin E and cucurbitacin E glycoside, which are the most common cucurbitacins identified in food plants, have oral LD₅₀ values in mice of 340 and 40 mg/kg body weight, respectively. The main toxicological effect produced by cucurbitacin D in experimental animals appears to be an increase in capillary permeability, irritation of the intestinal mucosa, and a strongly increased intestinal motility. The animals die from congestion of the intestine, pancreas, liver and kidneys. The lungs become strongly oedematous and contain large amounts of fluids. Fluids are sometimes present in appreciable amounts also in the thoracic and abdominal cavities.

Although chronic exposure to cucurbitacins is highly unlikely in humans, such exposure of experimental or domestic animals results in pronounced toxicity. Severe diarrhoea, reduced haemoglobin level, haematocrit and red blood cell count were observed in surviving mice fed 0.05 % cucurbitacins for 10 weeks. Sheep and goats fed fresh plant material containing cucurbitacins died with catarrhal enteritis of the intestine and hepatic fatty changes. Chickens fed cucurbitacin-containing plant material had damaged intestine and liver.

Very few data are available on humans intoxicated by cucurbitacins. It is known, however, that irritation of the mucous membranes manifested as oedema of the pharynx, dyspnoea, drooling, dysphagia, and vomiting, occur within minutes after oral exposure to preparations containing cucurbitacins. In general the cucurbitacin content of products that have resulted in intoxication of persons is unknown. In 1982 the Australian Department of Health reported more than 20 cases of intoxication in Queensland after consumption of zucchini squash. The symptoms included stomach cramps, diarrhoea, vomiting and headaches. Symptoms like severe cramps, persistent diarrhoea and collapse have been reported within 1 to 2 hours after a single ingestion of as little as about 3 g bitter zucchini.

In the Nordic countries reports on poisoning from bitter zucchini (squash) occurred in Denmark in 1995 and 1996 and in Sweden in 1996.

1.2 Svensk Sammandrag (Swedish Summary)

Rapporten syftar till att sammanställa vår kunskap om förekomsten av cucurbitaciner i vegetabilier tillhörande gurkväxternas familj (*Cucurbitaceae*) och beskriva de effekter som ämnena kan ge upphov till när gurkväxterna konsumeras.

Cucurbitaciner är tetracykliska triterpener med ett cucurbitanskelett som gemensam grundstruktur. De skiljer sig från andra tetracykliska triterpener genom att de är omättade och innehåller ett flertal keto-, hydroxy- och ättiksyragrupper. Sinsemellan särskiljs de olika cucurbitacinerna med bokstäver, kemiskt karakteriseras de av olika sidogrupper och olika position och antal av dubbelbindningarna. Cucurbitacin A är en tidigt identifierad substans, medan cucurbitacin L identifierades relativt nyligen.

Det är ännu inte säkerställt om cucurbitacinerna förekommer som sådana i växten eller om de förekommer kopplade till sockermolekyler och att dessa extremt lätt spjälkas av från cucurbitacinskelettet av β -glykosidas som aktiveras när växtcellerna förstörs vid preparation inför den kemiska analysen. I den glykosylerade formen uppträder cucurbitacinerna vanligen som β -2-monoglykosider där sockerdelen antingen är D-glykos eller L-rhamnos.

Cucurbitaciner är ytterst bittra ämnen som dessutom är mycket giftiga. Över 100 av de 900 kända arterna i gurkväxternas familj innehåller dessa ämnen. Den högsta koncentrationen finner man normalt i växternas rötter. Det är dock inte rötterna, utan frukterna, som normalt används som föda. Rötterna har dock tidigare använts inom traditionell medicin. Till viss del har de fortfarande denna användning. Bland växter har cucurbitaciner endast påvisats i gurkväxternas familj *Cucurbitaceae*.

Frukt från odlade gurkväxter som gurka, squash och andra pumpa-sorter, kalebass, melon och vattenmelon har alla förädlats för att bli av med cucurbitacinerna. Man tror att de förädlade sorterna bär ett arvsanlag (en mutation) som undertrycker produktionen av cucurbitaciner. Eftersom återmutationer kan uppkomma slumpmässigt kan det uppträda plantor som bär bittra och giftiga frukter på grund av deras innehåll av cucurbitaciner. Vattenmelon och squash som återfått förmågan att producera cucurbitaciner har rapporterats innehålla mellan 930 och 3100 mg/kg cucurbitacin E. En viss del av fröna bildade av sådana plantor bär anlaget att kunna bilda cucurbitaciner. 'Chayote' och 'snake gourds' är gurkväxter som odlas och konsumeras i andra delar av världen men som aldrig visats producera några bittra frukter. Däremot innehåller de unga plantor-

na (skotten) cucurbitaciner, vilket visar att växten bär på arvsanlagen som styr bildningen av cucurbitaciner men att dessa inte uttrycks i frukterna.

I de nordiska länderna sker den enda exponeringen för cucurbitacin via födan om man konsumerar frukter från återmuterade gurkväxter. Normalt saknar gurkväxterna dessa ämnen och är söta. I de enstaka fall då en återmutation givit tillbaka gurkväxten förmågan att bilda cucurbitaciner blir frukten extremt bitter. Människan kan känna igen den bittra smaken hos cucurbitacin E glykosiden (den minst bittra av de testade föreningarna) i mosad squash redan vid en nivå av 2 ppm (parts per million, motsvarande 2 mg/kg). Om en person tar en munsbit av squash eller vattenmelon (cirka 20 g) och denna innehåller mellan 930 och 3100 mg/kg cucurbitaciner, exponeras personen för cirka 18–60 mg cucurbitaciner. Detta motsvarar 0.3–0.9 mg /kg kroppsvikt hos en vuxen person på 70 kg. Högre exponeringar är osannolika eftersom man med största sannolikhet reagerar på den bittra smaken. Kronisk exponering för cucurbitaciner kan inte förväntas dels på grund av att återmutationerna som ger förmåga att producera cucurbitaciner uppkommer slumpmässig, dels på grund av att ämnet ger en extremt bitter och avskyvärd smak.

Det finns ingen tillgänglig information om absorption av cucurbitaciner från mag-tarmkanalen, metabolism i kroppen och utsöndring av föreningarna via urinen eller avföringen. De toxiska effekter som rapporterats hos djur och människor indikerar dock att dessa föreningar absorberas till en viss grad.

Exponering av försöksdjur för cucurbitaciner ge upphov till akut förgiftning. Även om oral exponering ger mindre allvarliga toxiska effekter än exponering via injektion under huden, i magen eller i blodbanorna, är den orala dosen som dödar 50% av försöksdjuren (LD_{50} hos möss) så låg som 5 mg/kg kroppsvikt för vissa cucurbitaciner (cucurbitacin D och I). De mest vanliga cucurbitacinerna i grönsaker, cucurbitacin E och cucurbitacin E glykosid, är mindre akutgiftiga och har orala LD_{50} - värden i möss på 340 respektive 40 mg/kg kroppsvikt. De påtagligaste effekterna av cucurbitacin D (en av de mest studerade cucurbitacinerna) tycks vara att de ger en förhöjd genomsläpplighet hos kapillärer, irritation av tarmens slemhinna (mucosan) och starkt ökad tarmrörelse. Djuren dör av stockningar i flera organ såsom i tarmen, bukspottsörteln, levern och njurarna. Vätska ansamlas i lungorna som blir starkt ödemiska. Avsevärda mängder vätska ansamlas även i bröst- och bukhålan.

Även om kronisk exponering för cucurbitaciner är högst osannolikt hos människa, resulterar sådan exponering hos försöksdjur och husdjur i förgiftning. Svåra diarréer och reducerade nivåer av hemoglobin, hematokrit och röda blodkroppar observerades hos möss som överlevde utfodring med 0.05% cucurbitaciner under 10 veckor. Får och getter matare med färskt foder av cucurbitacin-innehållande växter avled och befanns ha tarmkatarr och fettförändringar i levern. Kycklingar utfodrade

med cucurbitacininnehållande växter utvecklade skador på tarmen och levern.

Vi har mycket få data på människor som förgiftats av cucurbitaciner. Det är emellertid känt att irritation i slemhinnorna – manifesterade som ödem i svalget, andnöd, svårighet att svälja, drägglande och kräkningar – uppträder inom några minuter efter oral exponering av preparat som innehåller cucurbitaciner. Oftast saknas uppgift om cucurbitacininnehållet hos de produkter som givit upphov till förgiftning. År 1982 rapporterade det australiensiska hälsodepartementet att mer än 20 personer från Queensland förgiftats efter att ha konsumerat zucchini-squash. Symptomen var magkramper, diarré, kräkningar och huvudvärk. Svår kramp, ihärdig diarré och kolapps har rapporterats uppträda 1 till 2 timmar efter ett så lågt enskilt intag som 3g bitter zucchini.

I Norden har förgiftning efter konsumtion av bitter zucchini rapporterats i Danmark 1995 och 1996, samt i Sverige 1996 och 2004.

2. Introduction

Cucurbitacins are triterpenoids originally identified in cucurbitaceous plants, and whose toxic potential was first described in 1932 and then came into focus in 1981 when a highly toxic and extremely bitter compound was identified in zucchini squash canned in California (Steyn, 1932; Rymal *et al.*, 1984). High quantities of the same substance were subsequently found also in another squash variety cultivated in two regions of South-eastern United States. The compound was identified as cucurbitacin E. In the course of the following year – November 1981 to December 1982 – 22 cases of severe food poisoning with commercially produced zucchini were reported from Queensland, Australia (Ferguson *et al.*, 1983; Herrington, 1983).

Reports on poisonings with plants in the Nordic countries are more recent. In 1995 and 1996 bitter squash (zucchini, courgette) were notified to the Danish Food Administration several times (Pilegaard and Søborg, 1995; Gry, personal communication) and in some of those intoxications occurred. The Danish authorities found it difficult to advise the market and the consumers as no comprehensive review on the toxicity of the bitter principle was available, and food items from these plant species are generally “sweet” or “non-bitter”. Cases of intoxication have also occurred in Sweden in 1996 and 2004 (Andersson, personal communication).

This report aims to review the occurrence of bitter cucurbitacin derivatives in fruits of plants, and describe the hazard connected with their occurrence in these plants. It also aims to collect background information of importance for risk assessment of genetically modified cucurbitaceous plants. The report does not review the occurrence of cucurbitacins in other types of organisms, and do not consider protective effects related to medicinal use of cucurbitacin-containing plants, if such effects has been identified *in vitro* or *in vivo*.

3. Identity, physical and chemical properties, analytical methods

The first cucurbitacin was isolated as a crystalline substance already in 1831 and given the trivial name α -elaterin. Attempts to determine its chemical structure were undertaken in the beginning of the twentieth century, but success waited until the 1960's. Early studies have been reviewed by Hegnauer (1957). The description of the chemical structure was the breakthrough that triggered the identification of cucurbitacins both in the *Cucurbitaceae* family and in other plant families (Lavie and Glotter, 1971). Cucurbitacins are tetracyclic triterpenes with a cucurbitane skeleton, Figure 1. The cucurbitacins differ from most other tetracyclic triterpenes by being highly unsaturated and containing numerous keto-, hydroxy- and acetoxy-groups (Table 1).

Cucurbitacins may be isolated as free "aglycones" or as monoglycosylated compounds, the saccharide unit being D-glucose or L-rhamnose. Most plants shown to contain cucurbitacin glycosides also contain highly active β -glucosidases (elaterases). Elaterases have been found in several plant genera of *Cucurbitaceae*, including *Cucumis* and *Lagenaria*, whereas it has not yet been detected in other genera such as *Citrullus* and *Cucurbita* (Enslin and Rivett, 1957). This could be a matter of lack of investigations as cucurbitacine glycosides have been found in for example *Citrullus lanatus*, *Citrullus vulgaris*, and *Cucurbita pepo*. When the plant tissue is damaged the β -glucosidase will reach and cleave its substrate and rapidly release the aglycone. It is not clear to what extent free cucurbitacins occur in intact plants (Teuscher and Lindequist, 1994). To a large extent, they may very well be produced by the action of β -glucosidases when plant materials are processed for chemical analysis. The following sections of this chapter focus on the chemistry, nomenclature and physical and chemical properties of cucurbitacin aglycones.

3.1 Identity

The basic structure of triterpenes is built from six isoprene units. Triterpenes are accordingly C_{30} -compounds. Cucurbitacins are derivatives of the hypothetical triterpene hydrocarbon cucurbitane (IUPAC name 19(10-9 β)-abeo-5 α -lanostane; Figure 1), which when modified by oxygen containing substituents and double bonds produce the various cucurbitacins. A number of commonly occurring cucurbitacins are described in Table 1 (Teuscher and Lindequist, 1994) and illustrated in Figure 2. Cu-

cucurbitacin glycosides (usually) have the saccharide linked to carbon atom 2 (2-O- β -glycosides).

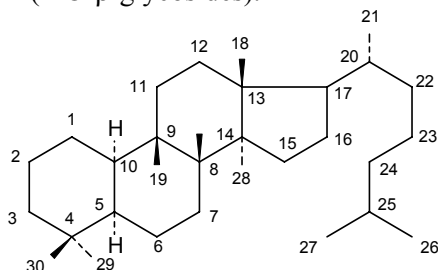


Fig. 1 *Cucurbitane skeleton.*

With exception of cucurbitacin A, J, and K, the cucurbitacins listed in Table 1 have all been found in foods from plants of the *Cucurbitaceae* family. The most common cucurbitacins in the *Cucurbitaceae* family are cucurbitacin B and D (Miró, 1995).

A special group of cucurbitacins is called momordicosides after their occurrence in *Momordica charantia*. Momordicosides have never been identified in any other plant species. Although *M. charantia* has been reported to be toxic to humans and animals, this plant is in other areas of the world grown as a vegetable and herb, as well as for its medicinal properties. The common feature of momordicosides is that C₁₉ has been oxidised to an aldehyde group. The structural formulas of the most important momordicosides are shown in Figure 3.

During the last 30 years a large number of cucurbitacins have been isolated, also from various plant species belonging to other plant families than *Cucurbitaceae*, and their structure elucidated. The driving force for this development has been the search for plant preparations active against various forms of disease. Many of the plant species harbouring these compounds were identified in cytotoxicity tests where the plant was screened together with other plants, because of their reputed effects in herbal remedies against various neoplastic disorders. In these studies they were found to have similar cytotoxic profiles as plants of the *Cucurbitaceae* family containing cucurbitacins. Cucurbitacins of non-food plants are not generally dealt with in this report.

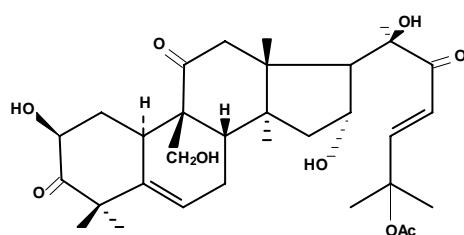
3.2 Nomenclature

Cucurbitacin aglycones isolated from various species of plants have been given the general name cucurbitacin (rarely cucurbitacine) (Enslin, 1954). The different cucurbitacins have been specified by letters from A to T, the further down the alphabet the later the cucurbitacin has been identified. Some cucurbitacins are also known by their trivial name. The first cucurbitacin isolated as a crystalline substance, elaterin, was not structurally described and shown to be a cucurbitacin until after a few other

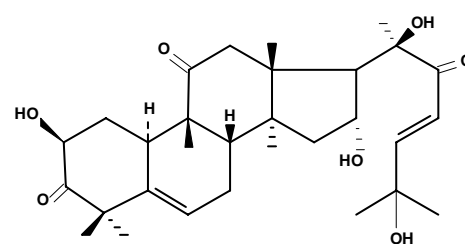
cucurbitacins had been described. Therefore, elaterin is now known under the name cucurbitacin E. Rarely used names for cucurbitacin D and cucurbitacin I are elatericin A and elatericin B, respectively.

Table 1. Chemical modification of cucurbitane (19(10-9 β)-abeo-5 α -lanostane) resulting in various cucurbitacins.

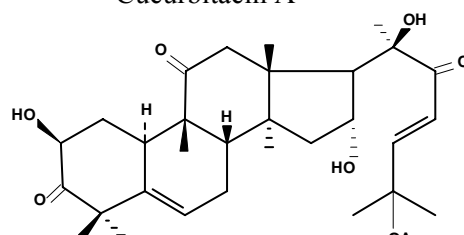
Name	Chemical group on carbon						Double bonds				
	2	3	11	16	19	20	22	24	25		
Cucurbitacin A	OH $_{\beta}$	=O	=O	OH $_{\alpha}$	OH	OH $_{\beta}$	=O		OAc	Δ^5	Δ^{23}
Cucurbitacin B	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O		OAc	Δ^5	Δ^{23}
Cucurbitacin C		OH	=O	OH $_{\alpha}$	OH	OH $_{\beta}$	=O		OAc	Δ^5	Δ^{23}
Cucurbitacin D	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O		OH	Δ^5	Δ^{23}
Cucurbitacin E	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O		OAc	Δ^1	Δ^5
Cucurbitacin I	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O		OH	Δ^1	Δ^5
Cucurbitacin J	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O	OH $_{\alpha}$	OH	Δ^1	Δ^5
Cucurbitacin K	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O	OH $_{\beta}$	OH	Δ^1	Δ^5
Cucurbitacin L	OH $_{\beta}$	=O	=O	OH $_{\alpha}$		OH $_{\beta}$	=O		OH	Δ^1	Δ^5



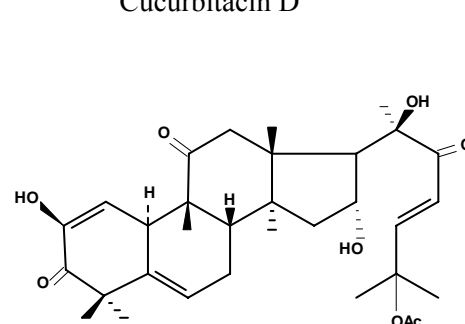
Cucurbitacin A



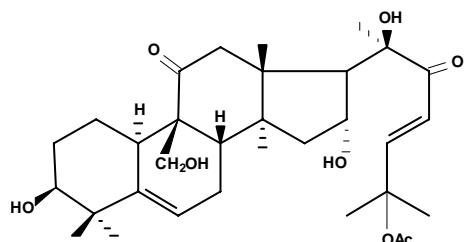
Cucurbitacin D



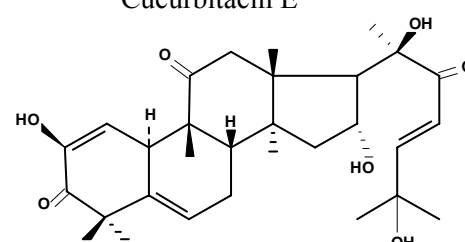
Cucurbitacin B



Cucurbitacin E



Cucurbitacin C



Cucurbitacin I

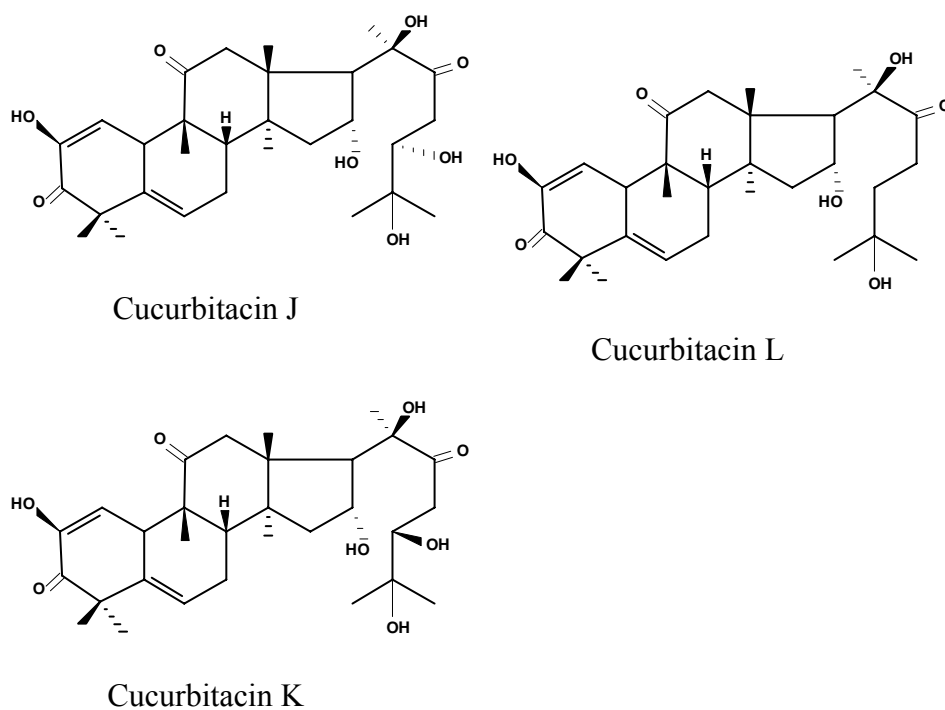
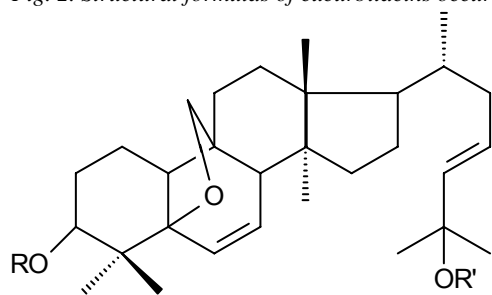


Fig. 2. Structural formulas of cucurbitacins occurring in food plants.



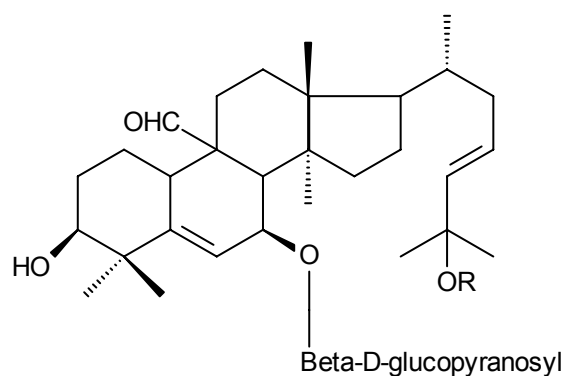
Momordicosides

F₁ R= β-D-glucopyranosyl R'= CH₃

F₂ R= β-D-allopyranosyl R'= H

G R= β-D-allopyranosyl R'= CH₃

I R= β-D-glucopyranosyl R'= H



Momordicosides

K R= CH₃
L R= H

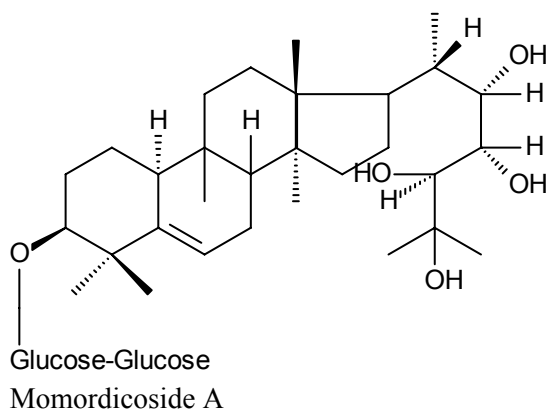


Fig. 3. Structural formulas of selected momordicosides.

3.3 Physical and chemical properties

At room temperature cucurbitacins are generally crystalline substances. Most cucurbitacins are soluble in petroleum ether, chloroform, benzene, ethyl acetate, methanol and ethanol, but are insoluble in ether. They are only slightly soluble in water. The compounds usually have absorption maxima for ultraviolet light between 228 and 234 nm.

The chemically reactive positions of the cucurbitacin molecules are the many ketone and alcohol groups present.

3.4 Analytical methods

Cucurbitacins are generally extracted from plant material with methanol or ethanol. The aglycones have a low solubility in water, but significant solubility in chloroform. Partition between these two solvents is frequently used to partially purify cucurbitacins from alcoholic plant extracts. Purification of extracts have generally been performed by open-column chromatography on silica gel, alumina or florisil, or by thin layer chromatography with the same media (Dinan *et al.*, 2001). The various cucurbitacins have earlier been identified by a biological test using beetle feeding and specific colour reactions. This method has been exchanged by thin layer chromatography, high performance liquid chromatography (HPLC), and mass spectrometry (MS) (Hutt and Herrington, 1985), or a combination of these methods. The more recent structure elucidations of cucurbitacins have been performed with MS- and NMR-based methods (Huang *et al.*, 1988; Muñoz *et al.*, 2000), while routine quantitative analyses are usually performed with HPLC methods (Matsuo *et al.*, 1999). A method using reverse phase thin layer and high performance liquid chromatography has been developed to determine specifically the level of cucurbitacin E glycosides in bitter squash (Hutt and Herrington, 1985).

In growing plants cucurbitacins are generally present as glycosides (as 2- β -*O*-glucosides). These are frequently hydrolysed by elaterase before extraction to give the aglycone. The free cucurbitacins occur naturally in seeds (Dinan *et al.*, 2001). An HPLC-MS method (electrospray-ionisation (ESI) or atmospheric pressure chemical ionisation (APCI)) based on a C18 column and a gradient of acetonitrile in 0.01% trifluoroacetic acid was recently developed to increase selectivity of the analytical technique by reducing complications arising from interfering substances and allowing simultaneous analysis of aglycones and glycosides in the plant extracts (Sturm and Stuppner, 2000).

3.5 Organoleptic properties

Cucurbitacins are immensely bitter. Taste panels have recognised down to 1 ppb cucurbitacin B and down to 10 ppb cucurbitacin E glycoside in water (Metcalf *et al.* 1980). In another study the test panel required 1 ppm of cucurbitacin E glycoside in water and 2 ppm cucurbitacin E glycoside when mixed with squash pulp to recognise the bitterness (Hutt and Herrington, 1985).

4. Biosynthesis

Balliano *et al.* (1983) have reviewed the biosynthesis of cucurbitacin glycosides from squalene-2,3-epoxide to the final cucurbitacin, pointing out the possible routes for biosynthesis (see Figure 4): “It has been suggested that cucurbitacins are biosynthesised from squalene-2,3-epoxide (**1**), through cyclization to the cat ion, **2**, and subsequent transformation of a lanostane C-9 carbonium ion, **3**, which could follow different routes (*a*, *b*, and *c* in Figure 4). Loss of the C-11 proton from **3** (route *a*, figure 4) could give parkeol (**4**) a tetracyclic triterpene found in *Butyrospermum parkii*. The migration of the C-10 methyl to C-9, induced by an electron deficiency at C-9, followed by hydrogen migration (H-5 → H-10) and elimination of a C-6 proton, will give the cucurbitane skeleton **5** (cucurbita-5,24-dienol). Alternatively (route *b*), loss of a proton from the C-19 methyl group with closure of the 9 β ,19-cyclopropane ring, could give cycloartenol (**6**), the supposed general precursor of phytosterols. Such an intermediate as **6**, by opening of the 9 β ,19-cyclopropane ring in concert with hydrogen migration and proton elimination, could lead to **5**. The third possibility (route *c*) invokes the direct intermediary of cucurbita-5,24-dienol (**5**), the simplest tetracyclic triterpene with a cucurbitane skeleton, present both in seed oil of gourd (*Lagenaria leucantha* var. Gourda) and in *Bryonia dioica* seedlings. Cucurbita-5,24-dienol (**5**) could be produced directly from **3** by multiple migration (Me-10 → Me-9, H-5 → H-10) together with the final elimination of the C-6 proton”.

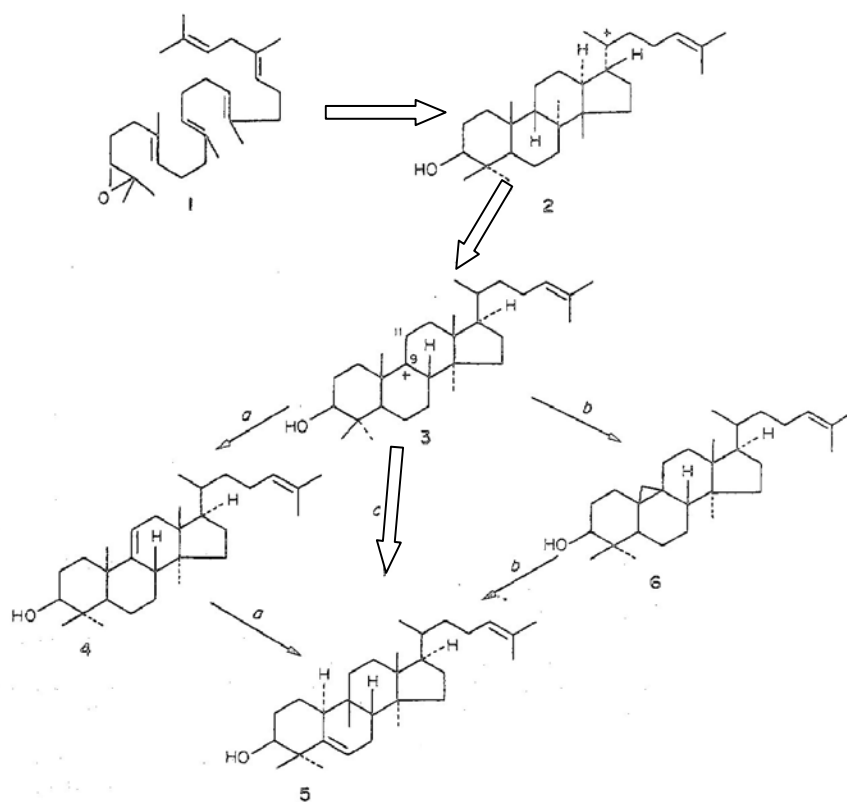


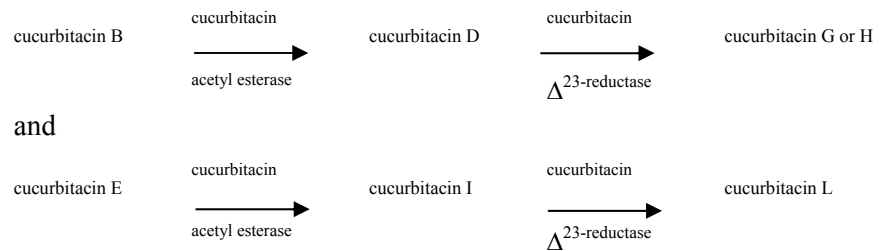
Figure 4. Proposed biosynthesis of cucurbita-5,24-dienol (5), a precursor for the cucurbitacins (from Balliano *et al.*, 1983).

Which of the routes, *a*, *b*, or *c* in figure 4, is most probable for the conversion of the lanostane C-9 carbonium ion (3) to cucurbita-5,24-dienol was investigated in tracing studies with radiolabelled precursors. Labelled cucurbitacin C was unambiguously detected following *in vivo* incubation of *Cucumis sativus* seedlings with [2-³H]10 α -cucurbita-5,24-dien-3 β -ol (abbreviated cucurbita-5,24-dienol (5)). By contrast, under the same conditions [2-³H]cycloartenol (6), the precursor of phytosterols gave only the expected 4-desmethyl sterols, and [2-³H]parkeol (4) was recovered unaltered. This substantiated the route *c* in Figure 4, excluding parkeol or cycloartenol as intermediaries. These studies support the existence in *Cucurbitaceae* of an enzyme, capable of directly converting squalene-2,3-epoxide into cucurbita-5,24-dienol (Balliano *et al.*, 1983).

Akihisa *et al.* (1986) subsequently demonstrated the widespread occurrence of 10 α -cucurbita-5,24-dien-3 β -ol in many seeds (and some mature plant parts) of food plants. This compound, being synthesised directly from squalene-2,3-oxide, is now considered an important intermediate in the biogenesis of cucurbitacins. The fact that two pentacyclic compounds, glutinol and simiarenol (both Δ^5 -unsaturated and 3 β -monohydroxy triterpenes like cucurbita-5,24-dienol), are frequently

found together with cucurbita-5,24-dienol is taken as support for the biosynthetic route of triterpenoid compounds illustrated in Figure 4.

The primary cucurbitacins formed in *Cucurbitaceae* plants are cucurbitacin B and E. Other cucurbitacins could have been produced from the primary cucurbitacins by the action of acetyl esterases, and these cucurbitacins could be further modified by a specific cucurbitacin Δ^{23} -reductase having the primary cucurbitacins and their derivatives as substrates. Such an enzyme has been isolated from *Cucurbita maxima* (Schabort and Potgieter, 1968; Schabort and Teijema, 1968; Schabort *et al.*, 1968). The hypothesis was verified by tests, supporting the following sequential route of production of cucurbitacins (Rehm and Wessels, 1957):



5. Occurrence

Cucurbitacins are found in many cucurbitaceous plants – they have been demonstrated in some 100 of the about 900 species belonging to this family. They are most common in species of the *Bryonia*, *Cucumis* (cucumber, melons), *Cucurbita* (squash, pumpkin), *Luffa* (angled gourd), *Coccoloba*, *Echinocystis*, *Lagenaria* (bottle gourd) and *Citrullus* (watermelon, colocynth) genera, and in the squirting cucumber, *Echballium elaterium*. The cucurbitacins are responsible for the bitter taste of these plants (Teuscher and Lindequist, 1994). Plants of the genera *Momordica* contain a special group of cucurbitacins called momordicosides (see Figure 3). The most important species of this plant genus is the bitter gourd, *Momordica charantia*, also called bitter cucumber or balsam pear.

The level of cucurbitacins often varies between tissues. They may be concentrated in fruits and roots of mature plants but only produce slightly bitter leaves and stems. In many plant species, the roots are the only bitter tissue. With few exceptions, bitter roots appear to be a prerequisite for bitterness in other parts of the plant. However, the occurrence of cucurbitacin in vegetative parts is not a predictor for the occurrence of this class of compounds in the fruits (Rymal *et al.*, 1984).

Due to their economic importance, fruits are by far the plant part most often analysed for cucurbitacins. In fruits where cucurbitacins are produced, they normally reach their highest concentration by maturity, though a few exceptions are known. Seeds generally contain very low concentrations of cucurbitacins.

Cucurbitacin-producing plants have also been identified outside the *Cucurbitaceae* family. Among these are, for example, various *Gratiola* species (*Scrophulariaceae*), *Begonia tuberhybrida* (*Begoniaceae*), *Anagallis arvensis* (*Primulaceae*), *Phormium tenax* (*Liliaceae*), *Tropaeolum majus* (*Tropaeolaceae*), and *Purshia tridentata* (*Rosaceae*). The seeds of certain cruciferous plants, like various *Iberis* species and *Lepidium sativum* also contain cucurbitacins (Teuscher and Lindequist, 1994).

5.1 Food plants and their accidental contents of bitter and toxic principles

5.1.1 *Cucurbitacins*

Because of the extreme bitterness of cucurbitacins, plants containing such compounds would generally not be consumed. Due to the original selection of non-bitter plants for food use by our ancestors and later on by plant breeders, all parts of cucurbitaceous plants intended for food use are nowadays generally free of cucurbitacins. After searching around 15 000 cucumber plants for non-bitterness of seedlings, Andeweg and De Bruyn (1959) found one American variety of cucumber, Improved Long Green, which did not show any sign of containing bitter compounds and had no bitter taste. F₁ plants from a cross between non-bitter Improved Long Green and a bitter Dutch variety were all bitter, whereas selfing of the non-bitter Improved Long Green plant only produced seeds giving non-bitter seedlings. The F₂ seeds produced by selfing the bitter hybrid between Improved Long Green and the Dutch bitter variety produced non-bitter and bitter seedlings in the proportion 3:1. A similar monohybrid cleavage of the bitterness trait has been observed in melon plants produced from F₂ seeds obtained in selfing of a hybrid between Westlandse Enkele Net (bitter seedlings) and Hales Best 36 (non-bitter seedlings). Thus, bitterness seems to be controlled by a dominant gene. Similar observations indicating a dominant gene determining bitterness have been obtained also from studies on Wild Hanzil Medicinal cucumber of India (Barham, 1953, 1954), the fruits of *Lagenaria leucantha* (Pathak and Singh, 1949, 1950), the fruits of *Cucurbita pepo* (Grebenščíkov, 1954, 1955), the fruits of *Citrullus vulgaris* (Arasimovic, 1937), and the fruits of *Citrullus colocynthis* (Shimotsuma and Ogawa, 1960).

However, Rehm and Wessels (1957) found that non-bitter fruits in some cases are produced by bitter plants, indicating that the plant had the gene for bitterness, but that other factors controlled fruit bitterness. Thus, the original hypothesis of a single dominant gene must in many cases be too simplistic. Enslin and Rehm (1958) found a gene suppressing fruit bitterness in *Citrullus lanatus*. The presence of two copies of the recessive suppressor gene would render the fruit bitter (Chambliss *et al.*, 1968). To what extent there is a difference in cucurbitacin content between plants heterozygous for the suppressor gene and plants homozygous for the allele unable to suppress fruit bitterness is not known. The potential to synthesize cucurbitacins in cucurbitaceous food plants is however demonstrated by the widespread occurrence in cucurbitaceous food plants of the cucurbitacine precursor cucurbita-5,24-dienol (10 α -cucurbita-5,24-dien-3 β -ol) in seeds and some mature plant parts (Akihisa *et al.*, 1986). When back mutations from non-bitter to bitter plants occur, plant parts or progeny are produced that can synthesize the bitter and toxic compounds.

Fruits produced by such plants have been shown to contain very high amounts of cucurbitacin E (Table 2). In addition, low amounts of cucurbitacin C have been quantified. A fraction of the offspring of such plants will also carry the genetic information required to produce cucurbitacins – see section on squash/zucchini (*Cucurbita pepo*).

Because of the unpredictable occurrence of cucurbitacin-producing food plants, no average content of cucurbitacins in the food we consume can be given. The quantitative information in Table 2 instead gives the cucurbitacin content reported in accidentally occurring bitter fruits. From this table it can be concluded that it is cucurbitacin C and E, and their glycosides, that are most interesting in the context of food plants.

Although fruits of consumed cucurbitaceous plants usually are void of cucurbitacins, these substances may occur in seedlings of the same plants. Using a semi-quantitative analytical method for cucurbitacins, Rehm and Wessels (1957) measured the cucurbitacin content of radicles and cotyledons of ordinary food plants. The data of this investigation are shown in Table 3. It is evident that a wider spectrum of cucurbitacins is found in seedlings of food plants than in fruits of the corresponding plant.

Table 2. Reported accidental contents of cucurbitacins in food plants.

Plant Scientific name	Plant Common name	Cultivar, etc.	Plant part	Cucurbitacin identi- fied	Amount repor- ted mg/kg fresh weight	Reference
<i>Citrullus lanatus</i>	Watermelon	Warpaint Hybrid- form	Fruit	Cucurbitacin E glycoside	480	Herrington <i>et al.</i> , 1986
<i>Citrullus lanatus</i>	Watermelon	Hawkesbury Mutant	Fruit	Cucurbitacin E glycoside	1500–2100	Chambliss <i>et al.</i> , 1968
<i>Citrullus vulgaris</i>	Watermelon	Accession 242 Wild form	Fruit	Cucurbitacin E glycoside	240 – 590	Chambliss <i>et al.</i> , 1968
<i>Cucumis sativus</i>	Cucumber	Bitter cultivated cucumber var.	Fruit	Cucurbitacin C	300	Enslin, 1954
<i>Cucumis sativus</i>	Cucumber	“sweet” form	Fruit	Cucurbitacin E	Identified	Guha and Sen, 1975
<i>Cucumis sativus</i>	Cucumber	“bitter” var. Hanzil	Fruit	Cucurbitacin C	Identified	Guha and Sen, 1975
<i>Cucurbita pepo</i>	Squash	Yellow straight- neck	Fruit/ market	Cucurbitacin E	Average – 3100	Rymal <i>et al.</i> , 1984
<i>Cucurbita pepo</i>	Squash	Yellow straight- neck	Fruit/ canned	Cucurbitacin E	Can content average – 930	Rymal <i>et al.</i> , 1984
<i>Cucurbita pepo</i>	Squash	Castleverde	Fruit	Cucurbitacin E	Stem end – 7200 Central portion – 2700	Rymal <i>et al.</i> , 1984
<i>Cucurbita pepo</i>	Squash	Blackjack	Fruit	Cucurbitacin E glycoside	600	Hutt and Herrington, 1985
<i>Cucurbita pepo</i>	Squash	Blackjack	Fruit	Cucurbitacin E glycoside	1120	Ferguson <i>et al.</i> , 1983

The following text shortly summarises our knowledge on the occurrence of cucurbitacins in common food plants. The food plants are presented in alphabetical order:

Citrullus lanatus. The common watermelon and the pie melon are the cultured varieties of this species. The pie melon is a melon with firm texture and bland taste, occurring naturalised in certain parts of Australia.

In a field study on introgression (volunteer pollination), using the watermelon variety Warpaint as recipient parent (donor parent unknown), Herrington *et al.* (1986) reported that the hybridisation resulted in a plant producing bitter red-fleshed watermelons with a cucurbitacin E glycoside content of 480 mg/kg fresh fruit (Table 2).

Chambliss *et al.* (1968) found no cucurbitacin E glycoside in the watermelon fruits of the non-bitter cultivar Hawkesbury. A bitter Hawkesbury mutant, on the other hand, contained between 1500 and 2100 mg cucurbitacin E glycoside/kg fresh weight (Table 2). Hybrids (F₁) between the two types contained between 900 and 1600 mg cucurbitacin E glycoside/kg fresh weight. A segregation of the ability to produce cucurbitacin E glycoside was observed in the second (F₂) generation.

Table 3. Approximate cucurbitacin content (mg/kg fresh weight) of cucurbitaceous seedlings of food plants (from Rehm and Wessels, 1957).

Species	Cucurbitacins present in									
	Radicles				Cotyledons					
	B	D	E	I	B	C	D	E	I	
<i>Citrullus vulgaris</i> , Water melon, wild			**					**		
<i>Citrullus vulgaris</i> , Water melon			**					**		
<i>Cucumis melo</i> , Musk melon	**	tr			**		tr	tr		
<i>Cucumis sativus</i> , Cucumber var. Hanzil	**				**	*				
<i>Cucumis sativus</i> , Cucumber	**				**	*				
<i>Cucurbita maxima</i> , Squash ^a	**	tr	*		**		*	*		
<i>Cucurbita pepo</i> , Pumpkin ^a	tr		**	tr	**		*	*		
<i>Lagenaria siceraria</i> Bottle gourd	tr		**	tr	tr			**	tr	
<i>Luffa acutangula</i> Ridged gourd	**		**		*			**		

* amount of bitter principle 10 – 99 mg/kg; ** amount of bitter principle 100 – 999 mg/kg; tr amount of bitter principle less than 10 mg/kg

a. These names are quoted from the work of Rehm and Wessels, 1957, but are not in accordance with the present terminology where pumpkin is *Cucurbita maxima* and squash is *Cucurbita pepo*

The cucurbitacin E glycoside in the bitter Hawkesbury water melon mutant was fairly homogeneously distributed between different areas of the fruits. The heart contained 2100 mg/kg fresh weight, the seed cavity 1300 mg/kg, the red flesh further out from the centre 1300 mg/kg, the pink

flesh close to the rind 1900 mg/kg, the rind 2500 mg/kg and the exocarp 1500 mg/kg (Chambliss and Jones, 1966).

A wild-type form called Accession 242 contained a mean of 240 mg cucurbitacin E glycoside/kg fresh weight in 1963 and a mean of 590 mg/kg fresh weight in 1964. The correlation between fruit bitterness and cucurbitacin E glycoside content was good (Chambliss *et al.*, 1968).

Citrullus vulgaris. As shown in Table 3, seedlings of the wild type and the cultivated form of this watermelon plant contain similar amounts of cucurbitacin E in radicles and cotyledons (Rehm and Wessels, 1957). Fruits of the wild form contain 240–590 mg/kg fresh weight 2-O- β -glucoside of cucurbitacin E (elaterinide) (Table 2), whereas the cultivated form contain undetectable levels of the compound (Guha and Sen, 1975, Le Men *et al.*, 1969).

Cucumis melo. Musk melon seedlings were early shown to contain cucurbitacin B and traces of cucurbitacin D in radicles and cotyledons, and in addition trace amounts of cucurbitacin E in the cotyledons, Table 3 (Rehm and Wessels, 1957). No cucurbitacins have been found in the fruit flesh of the musk melon.

Cucumis metuliferus, jelly melon. This plant is indigenous to South Africa and occurs in bitter and non-bitter forms in the wild state. The non-bitter form has sometimes been cultivated (Enslin *et al.*, 1954).

Cucumis sativus, the cucumber, occurs in three different forms – varieties which have vegetative parts that always are bitter and produce fruits that may be bitter, varieties which always have bitter vegetative parts but fruits that even under unfavourable conditions do not turn bitter, and varieties which completely lack bitter principles both in the fruits and in the vegetative parts of the plant (Andeweg and De Bruyn, 1959). Rehm and Wessels (1957) found seedlings of bitter (Hanzil) and non-bitter strains to contain similar amounts of cucurbitacin B in radicles and cotyledons. Cotyledons contain small amounts of cucurbitacin C in addition to cucurbitacin B (Table 3). Rice and co-workers (1981) harvested cotyledons from seedlings of three different cucumber cultivars 3–5 days after germination and found two of them to have a bitter taste. The third cultivar, Eversweet, had no bitter taste. All three cultivars contained cucurbitacin C as the single cucurbitacin. The non-bitter Eversweet cotyledons, in addition, contained similar quantities of an unidentified compound. Rehm (1960) reported that leaves of both bitter (Hanzil) and “sweet” strains of the cultured cucumber contain approximately 10 mg cucurbitacin C/kg fresh weight, whereas Van Keulen (1981) found mature top leaves of 10 different cucumber varieties to contain between 130 and 1130 mg cucurbitacin C/kg fresh weight.

Not unexpectedly, the percentage of bitter fruits is higher in bitter than in non-bitter cucumber cultivars (Kano *et al.*, 1997). Enslin (1954) reported 300 mg cucurbitacin C/kg fresh weight in a bitter but unspecified variety of the cultivated cucumber (Table 2). Also Gaha and Sen (1975)

reported cucurbitaine C in fruits of a bitter cucumber variety (Hanzil). These investigators found cucurbitacin E in fruits of a “sweet” cucumber variety (Guha and Sen, 1975). No cucurbitacin glycosides have been found in the cucumber (Rehm *et al.*, 1957).

Studies performed during later years have shown that the agricultural production methods might influence the level of bitter compounds in the cucumber. Kano and co-workers (1997) studied the production of cucurbitacins during growth of the cucumber. Young vigorous *C. sativus* plants of a Japanese variety synthesised larger amounts of cucurbitacin C than older less vigorous plants. Bitter fruits were particularly common on the first lateral shoot, harvested earlier than they were on the second lateral region. The bitterness was strong in young fruit, but decreased with age and size. It was observed that total leaf nitrogen and amino acid nitrogen levels were higher in the bitter cultivars than in non-bitter cultivars, which could indicate that high nitrogen levels promotes nitrogen metabolism, favouring cucurbitacin synthesis (Kano *et al.*, 1999). Subsequently, Kano and Goto (2003) showed that the occurrence of bitter fruits were higher in plants of the cultivar Kagafutokyuri grown at lower air temperatures, in plants of the bitter lines and in plants cultivated with twice as much nitrogenous fertilization as usual, than in plants grown at higher temperatures, in plants of the non-bitter lines and in plants cultivated with the usual amount of nitrogenous fertilizers. Total nitrogen, amino acid nitrogen, and protein content and 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity were higher in leaves of plants that produced higher occurrence of bitter fruit, and were higher in the bitter fruit than in the non-bitter fruit.

In rare cases the occurrence of cucurbitacins attracts insects, more generally it limits the insects ability to feed on the plants. In cucumber (*Cucumis sativus*), cucurbitacin C production is coupled to bitter taste and susceptibility to cucumber beetles, which are known to feed on cucurbitacin-producing plants. On the other hand, production of bitter cucurbitacins renders cucumbers resistant to the spider mite. The gene *Bi* confers bitterness to the entire plant. The *bi* gene prevents the fruit from becoming bitter under stress. A second recessive gene for bitter-free foliage, *bi-2*, has been described by Wehner *et al.* (1998), and segregates independently from the *bi* gene. Testing of bitterness of cotyledons from dihaploids derived from the F1 generation of the cross between a bitter-free (*bi/bi*) line and a bitter dihaploid revealed an expected 1:1 monohybrid segregation. The concentration of cucurbitacin C in the leaves is an important parameter in spider mite resistance in cucumber – a significant negative correlation between spider mite survival and cucurbitacin C content of individual dihaploid lines has been demonstrated (Balkema-Boomstra *et al.*, 2003).

The occurrence of a simply inherited chemical polymorphism provided Barrett and Agrawal (2004) with the experimental system for investi-

gating how genotypes of the cucumber differing in chemical resistance to herbivory of the beet armyworm (*Spodoptera exigua*) are affected by environmental and ontogenic factors. It turned out that all factors influenced plant resistance to herbivory in a complex and yet not understood way.

Cucurbita maxima. Seedlings of the pumpkin were early shown to contain large quantities of cucurbitacin B and small amounts of cucurbitacin D and cucurbitacin E in radicles and cotyledons, Table 3 (Rehm and Wessels, 1957). There are no reports on cucurbitacin-containing bitter pumpkins.

The pumpkin, *C. maxima*, originates from temperate South America where it was domesticated from *C. andreana*, a species indigenous to Argentina and Uruguay. The earliest archaeological remains of pumpkins from households are from 1800 BC in Peru (Sauer, 1993). In contrast to the pumpkin cultivated today, the ancestor, *C. andreana*, has been shown to contain cucurbitacins B, D, E and I, and the 2-O- β -glucopyranosylcucurbitacin E, 2-O- β -glucopyranosylcucurbitacin I and 2-O- β -glucopyranosylcucurbitacin B (Halaweish and Tallamy, 1993).

Cucurbita pepo, squash. Bitter fruits have been reported from a number of varieties of this species, for example Little Gem. Bitterness is only rarely found in varieties such as Golden Custard and Long White Bush (Enslin et al., 1954). The first squash (zucchini) was domesticated in North America from wild *Cucurbita texana*, occurring in the south central USA, and *C. fraterna*, occurring in North-eastern Mexico. From archaeological excavations in Mexico, domestication can be dated back to about 8000 BC (Sauer, 1993). Squashes were introduced to Europe by returning Spanish explorers in the 1500's. The major bitter principle in seedling radicles of squash is cucurbitacin E. This tissue also contained trace amounts of cucurbitacin B and cucurbitacin I (Table 3). The cotyledons contain moderate concentration of cucurbitacin B and small quantities of cucurbitacin D and cucurbitacin E (Table 3). No cucurbitacin glycosides have been identified in the *C. pepo* strains investigated (Rehm et al., 1957).

From 1981 to 1982 there were in the United States reports of several human poisoning episodes (see section nine) from eating varieties of zucchini squash derived by traditional plant breeding practices. During the same period, and as a result of a quality control testing in a company, a large commercial pack of tomato sauce with squash was not marketed because the squash was found to be extremely bitter. Rymal and co-workers obtained bitter canned zucchini pieces (from California and Alabama) in tomato sauce directly from the canning industry, and identified the main bitter substance as cucurbitacin E (Rymal et al., 1984). The quantity of cucurbitacin E in the canned zucchini sample was 930 mg/kg can content (Table 2). Fresh yellow straightneck squash fruits were obtained from farms in Alabama that had produced fruits leading to intoxica-

tion. The fruits were found to contain 3100 mg cucurbitacin E/kg fresh fruit (Table 2). Frozen raw zucchini fruit pieces of the Castleverde cultivar (being responsible for another intoxication) were also analysed for cucurbitacin E and found to contain 7200 mg/kg of flesh at the stem end, and 2700 mg/kg in the central portion of the fruit, Table 2 (Rymal *et al.*, 1984).

Seeds from the same source of the Castleverde cultivar producing the bitter yellow squash were planted in a growth chamber. Cotyledons from over 300 seedlings developing from the seeds were tasted for bitterness but none were found. A sample of 20 seeds (two did not germinate) from a self-pollinated bitter zucchini squash fruit was planted in a greenhouse and seedlings grown to fruit-producing maturity. Thirteen of the 18 seedlings had bitter cotyledons and five were non-bitter. Self-pollinated fruit were obtained from 15 of the 18 seedlings. Of these 12 were bitter and three non-bitter (two of these three came from plants having bitter seedlings). These observations indicate that the bitter form had probably been produced either through a back mutation of a sweet variety to a more bitter variety (reminding of a more ancestral condition), or through hybridisation (outcross) of the cultivated sweet variety with a wild bitter species (Rymal *et al.*, 1984).

Two groups of investigators have analysed freeze-dried samples of bitter squash of the cultivar Blackjack from Australia for cucurbitacins. Whereas Hutt and Herrington (1985) found 600 mg cucurbitacin E glycoside per kg fresh fruit in their analysed sample (Hutt and Herrington, 1985), Ferguson *et al.* (1983) detected 1120 mg cucurbitacin E/kg fresh weight (assuming a loss of 90% moisture with drying) in their sample (Table 2).

Lagenaria siceraria. Strains of the bottle gourd with bitter fruit have been shown to contain 100 mg bitter principles/kg root, whereas strains with "sweet" fruit have roots without bitter principles (Rehm, 1960). Seedlings of the strain with bitter fruit contained cucurbitacin E and traces of cucurbitacin B and cucurbitacin I in both radicles and cotyledons (Table 3). Other studies have reported fruits, leaves and roots to contain cucurbitacin B and cucurbitacin D (Rehm *et al.*, 1957; Guha and Sen, 1975).

Luffa acutangula, luffa or ridged gourd. The flesh of this rarely cultivated plant is non-bitter, but the seeds contain a bitter principle (Enslin *et al.*, 1954). Seedlings of the ridged gourd were early reported to contain cucurbitacin B and cucurbitacin E in radicles and cotyledons (Table 3) (Rehm and Wessels, 1957), and later to contain cucurbitacin B in roots and seeds (Guha and Sen, 1975). Bitter fruits of the ridged gourd have not yet been analysed for the presence of cucurbitacins.

Sechium edule. There are no reports on bitter fruits of the chayote. However, seeds of this plant have been reported to contain the cucurbitacin precursor cucurbita-5,24-dienol (Akihisa *et al.*, 1986).

Trichosantes species are known as snake gourds. Fruits of the snake gourd have been shown to contain the cucurbitacin precursor cucurbita-5,24-dienol (Akihisa *et al.*, 1986), perhaps indicating the possibility for the plant to back-mutate and obtain the capacity to synthesise cucurbitacins.

5.1.2 Momordicosides

Momordica charantia. Different parts of the “bitter melon”, “bitter apple” or “balsam pear”, or extracts of these plant parts are used in a wide range of medicinal preparations. The unripe fruit (light green outside with white flesh), and sometimes the young leaves, are used in oriental cooking, whereas the ripe fruit (yellow or orange outside, with orange flesh and a bright red placenta to which the seeds are attached) is intensely bitter and reported to be toxic to humans and animals. Although there is a very limited use of *Momordica charantia* as food in our part of the world, some information on the momordicosides is given below.

Already in 1957 Rehm *et al.* reported the occurrence of a bitter principle in roots of *M. charantia* and two other *Momordica* species, but the investigators were unable to identify the bitter compounds. Nearly twenty years later, the seeds of this plant were suggested to contain cucurbitacin B and K but not even the investigators were convinced about their finding (Guha and Sen, 1975). Subsequently, Japanese workers isolated and identified two bitter cucurbitacins, momordicoside K and L, and 4 non-bitter cucurbitacins, momordicoside F₁, F₂, G and I in immature *M. charantia* fruits (Okabe *et al.*, 1982a, 1982b). The chemical structures of the momordicosides are shown in Figure 3. All the aglycones had the common feature of lacking the C₁₁ ketone-group present in ordinary cucurbitacins, instead having an aldehyde group at the C₁₉ position. The aglycones of the bitter momordicosides have retained the aldehyde group intact and differ only at C₂₅ where momordicoside K has a methoxy-group and momordicoside L a hydroxy-group. Like the bitter compounds, the non-bitter momordicosides had a hydroxy- or a methoxy-group at position C₂₅, but their aldehyde group at position 29 was exchanged for a tetrahydro-furano-ring from C₉ to C₅, and a Δ_6 double bond instead of the Δ_5 double bond present in cucurbitacins (Okabe *et al.*, 1982a, 1982b).

Wang *et al.* (2001) improved the analytical method and determined the amounts of momordicoside A in different tissues (seed and pulp) and varieties (long-white, large-white, and green-peel) of “bitter melon”. Remarkable differences in content were observed between varieties and tissues (approximately 500–1800 mg/kg d.w. in seeds, pulp <100 mg/kg d.w., and leaf extract non-detectable levels). However, nearly no variation in tissue level of momordicoside A was observed in any of the varieties when they were grown at various geographical localities. These observations indicate a strong influence of hereditary factors, and a very

modest influence of environmental factors on the momordicoside level in the "bitter melon" (Wang *et al.*, 2001).

5.2 Contents in other plants

A large number of traditional medicinal plants have been analysed for their chemical composition in the hope of identifying the active principle(s). Cucurbitacins have been looked for in many of the bitter medicinal plants, in particular if they belong to the Cucurbitaceae family. The biological activities of these cucurbitacins, including their pharmacological effects, have been summarized in the two small reviews available (Miró, 1995; Chen *et al.*, 2005). This review only gives information on some of the non-food plants studied.

5.2.1 Non food plants from the Cucurbitaceae family

Bryonia alba and *Bryonia dioica* contain cucurbitacins B, D, E, and I in the roots (Rehm, 1960). This observation has been confirmed by Konopa *et al.* (1974a), who in addition identified cucurbitacin J, K and L, and tetrahydrocucurbitacin I, and Pohlmann (1975) who in addition identified dihydrocucurbitacin E and dihydrocucurbitacin B in roots of both species. The amounts of cucurbitacins detected were dependent on the date of harvest, how long the material had been stored, and how the compounds had been extracted from the plants.

Caputo nigri. Twelve cucurbitacin glycosides, cabenosides A–L, have been isolated from the roots of cabaça-de-negro, a well-known Brazilian herb used in folk medicine. Three of the 12 glycosides had 29-norcucurbitacin as aglycone (ring A turned into a benzene-ring). The sugar moieties were situated at C₂, C₃, C₇ and/or C₂₅ (Nakano *et al.*, 1994, 1995a, 1995b).

Citrullus colocynthis. Historical records of an Egyptian physician from as early as 1522 BC, document the use of colocynth preparations (presumably an extract of *Citrullus colocynthis* – a wild species of the watermelon genus) as medicine (Bush-Brown, 1964). Pharmacologically these preparations have been used as purgatives (Belkin and Fitzgerald, 1952; Moore, 1910), anti-tumour agents (Power and Moore, 1909), and growth inhibitors for solid tumours *in vitro* and *in vivo* (Gitter *et al.*, 1961). The extremely high toxicity of these compounds has resulted in the abandonment of the pharmaceutical uses of the plant in recent years (Cassady and Suffness, 1980). Young leaves of the colocynth have a relatively low content of bitter principles, but old leaves and stems of this plant can contain as much as 1000–3000 mg cucurbitacins/kg plant material (Rehm, 1960). Recently, Seger and co-workers (2005) described 2-O- β -D-glucopyranosylcucurbitacins I, J, K and L in this plant.

Citrullus ecirrhosa is a perennial plant containing 0.9 % bitter principle in air-dried roots (Rehm, 1960).

Citrullus naudians is a perennial cucurbitaceous plant with extremely high concentrations of bitter principles in fresh roots, 1400 mg/kg fresh roots (Rehm, 1960). Varieties are known that produce bitter fruits, whereas other varieties produce non-bitter fruits. However, high amounts of bitter principles occur in fresh roots also of varieties producing not-bitter fruits (Rehm, 1960).

Cucumis asper and *Cucumis dinteri* are plants indigenous to South Africa. Both species contain high levels of cucurbitacins in leaves. Among the bitter principles, Rehm (1960) identified cucurbitacin M (1000 mg/kg) and cucurbitacin B (50 mg/kg) in *C. asper*, and cucurbitacin F (600 mg/kg) in *C. dinteri*.

Cucurbita foetidissima, the Buffalo gourd, is not used as food any more. Fruits and seeds were eaten by native Americans. The leaves, especially in spring, have a strong, disagreeable odour. The root can grow to enormous proportions – it can weigh up to 40 kg after three or four seasons. The de-fatted root powder has been found to contain 2000 mg cucurbitacin/kg whole root (Berry *et al.*, 1978). Similar amounts were detected in fresh roots by Rehm *et al.* (1957a).

Cyclanthera pedata. De Tommasi *et al.* (1996) isolated and identified six new cucurbitacin glycosides in the seeds of *C. pedata*. These compounds carried three types of aglycones; two were 29-nor-cucurbitacins, while the third was an ordinary cucurbitacin.

Ecballium elaterium. The squirting cucumber was the first cucurbitaceous plant from which a cucurbitacin was isolated. At that time, 1831, the compound was given the name elaterin (or α -elaterin, later identified as cucurbitacin E). The cucurbitacin E, B, D and I can be found in all plant tissues analysed (root, stem, leaf, flower, and fruit). Appreciable amount were found in the fruit but only trace amounts in the other tissues (Balbaa *et al.*, 1978). Until now a large number of cucurbitacin derivatives have been isolated from the fruit juice: cucurbitacin D, 22-deoxycucurbitacin D, cucurbitacin R, cucurbitacin L, cucurbitacin I, cucurbitacin B, cucurbitacin E, and a (23S,24Z)-16,23-epoxy cucurbitacin derivative (Seger *et al.*, 2004, 2005). Two cucurbitacin glycosides have been isolated and characterised from the fruit juice. The aglycones were cucurbitacin B and cucurbitacin D, the sugar moiety in both cases being glucose. Sezik (1997) analysed the cucurbitacin B content in fresh fruit juice samples by HPLC and found as much as 24 800 mg/kg cucurbitacin B. The fruit juice of the plant has a long history of use as a “drastic” purgative (Seifert and Elgamal, 1977). A case of soft palate and uvular oedema due to nasal administration of the fruit juice has been reported (Koussidis *et al.*, 2002). This plant species has lately been cultured *in vitro* as undifferentiated callus with the aim to produce cucurbitacins

(Attard and Scicluna-Spiteri, 2001). The highest yield of cucurbitacins yet obtained is 1.13% (w/w) cucurbitacin B (Toker et al., 2003).

Lagenaria leucantha. Generally known as bottle gourd, this plant has been cultivated for a very long time in India (Pathak and Sing, 1950). The flesh of the cultivated form differs from that of the wild form, the former being sweet and edible and the latter bitter and unpalatable. Genetical studies revealed that the bitter fruit taste was dependent on a single gene and that the allele for bitterness was dominant over the allele giving a sweet taste. The seed oil of this species has been shown to contain the cucurbitacin precursor cucurbita-5,24-dien-3 β -ol in the unsaponifiable matter (Itoh et al., 1980).

5.2.2 Non food plants from other plant families

Most plant species outside the *Cucurbitaceae* family that have been studied for their content of cucurbitacins seems to have been used as traditional plant medicines, often being recommended for use against fever, cancer, or inflammation. Many of the cucurbitacin-containing plants have been identified in a programme where the plants, or extracts of the plants, were screened for cytotoxicity in a battery of human tumour cell lines (totally 60 cell lines) obtained from the American National Cancer Institute. The cytotoxic principles were later isolated and identified and sometimes shown to be cucurbitacins. Only a few examples of these plants are given in this section. The examples contain identical or similar cucurbitacins to those naturally occurring in food plants.

Cruciferae.

Cucurbitacin B and cucurbitacin D have been isolated from *Iberis umbellata* seeds (Dinan et al., 1997a).

Euphorbiaceae.

Tessier and Paris (1978) demonstrated that the toxicity of the bark from three toxic African plants, *Maprounea africana*, *Maprounea membranacea* and *Spondianthus preussii*, was due to cucurbitacins (cucurbitacin A and 23,24-dihydro cucurbitacin A in *Maprounea*, and cucurbitacin E and cucurbitacin L in *Spondianthus*). The concentrations of cucurbitacin A in trunk bark from *Maprounea africana* was 45 mg/kg, whereas the total content of cucurbitacin A and 23,24-dihydro cucurbitacin A in the root bark of *Maprounea membranacea* was 10 mg/kg. The *Spondianthus* species contained 10 mg/kg cucurbitacin L and traces of cucurbitacin E in bark from the trunk.

Rosaceae

Cucurbitacin F, 15-oxo-cucurbitacin F and the 23,24-dihydrogen compounds of these cucurbitacins were isolated from twigs and leaves of *Cowania mexicana* (Konoshima *et al.*, 1993).

Aerial parts of a Chilean tree, *Kageneckia oblonga*, has been found to contain 3 β -(β -D-glucosyloxy)-16 α ,23 α -epoxycucurbita-5,24-dien-11 one, whereas seeds of another Chilean tree, *Kageneckia angustifolia*, contain cucurbitacin F and 2,3,16-triacetate-15-oxy-23,24-dihydroxycucurbitacin (Muñoz *et al.*, 2000, 2002). Toxicity studies *in vitro* have indicated that dihydrocucurbitacin F and 3 β -(β -D-glucosyloxy)-16 α ,23 α -epoxycucurbita-5,24-dien-11 one can be partly responsible for analgesic, antipyretic, and anti-inflammatory activity of *Kageneckia oblonga* extracts (Delporte *et al.*, 2002).

Scrophulariaceae.

Cucurbitacin E has been isolated from *Conoba scopaioides* (Musza *et al.*, 1994). Four cucurbitacin glycosides with a common aglycone, picfeltarraegenin, were isolated from *Picria fel-terrae*, a plant used in traditional Chinese medicine (Huang *et al.*, 1998).

5.3 Influence of storage and processing

A canned sample of bitter squash contained 930 mg cucurbitacin E per kg can content (Rymal *et al.*, 1984). This observation might indicate that cucurbitacin E is stable and not destroyed by the heat and the pressure treatment used during the canning process. As mentioned in section 3, it is not known whether the cucurbitacins only occur naturally as glycosides, or as aglycones or as a mixture of glycosides and aglycones in the different tissues of the plant. The information given by Rymal *et al.* (1984) is too limited to draw final conclusions about the possibility that the processing of the zucchini (squash) could have activated the endogenous β -glucosidase resulting in cleavage of the glycosidic bonds between the cucurbitacin aglycones and the saccharides.

6. Exposure estimation

Cucurbitaceous foods consumed in the Nordic countries are normally devoid of cucurbitacins. Thus, the exposure assessment with respect to cucurbitacins cannot make use of figures from production/import/export or consumption of these plants.

Due to the extremely bitter taste exposure of humans to cucurbitacins is rare and would in most cases be a single oral dose of one mouthful squash or watermelon (cucurbitacin content between 930 and 3100 mg/kg) that have regained the ability to produce cucurbitacins, which would correspond to 18–60 mg cucurbitacins from 20 g fruit. The exposure would in this example be between 0.3 and 1 mg/kg bw. Repeated or chronic exposure via food is not expected due to the random occurrence of cucurbitacin-containing plants and the extremely bitter and disagreeable taste of these plants.

7. Toxicokinetics

As indicated in previous sections, it is not clear whether cucurbitacins occur as glycosides and/or aglycones in the studied food plants. Upon processing of the food in the kitchen or during consumption of fresh plants, it is likely that cellular β -glycosidases of the plant food will come in contact with the cucurbitacin glycosides and cleave the glycosidic bond. If glycosides survive to the gastrointestinal tract, another possibility is that competent organisms of the gut microflora cleave the glycosidic bond.

There are no studies available on the absorption, distribution, metabolism and excretion of cucurbitacin glycosides, or cucurbitacin aglycones from bitter Cucurbitaceae in mammals. What is known in relation to bioavailability of cucurbitacins is that the time to appearance of toxic symptoms varies with the animal species used in the experiment, the route of administration of the compound, and the quantity that has been administered (Edery *et al.*, 1961).

8. Studies of toxicity

8.1 In vitro toxicity

A number of investigators have studied the cytotoxicity of cucurbitacin aglycones on various types of cells. Much less is known about the cytotoxicity of cucurbitacin glycosides. Often the aim of the studies has been to identify compounds with therapeutic value. The results of some of these investigations are summarised in Table 4.

It is evident from the data in Table 4 that cell growth of both prokaryotic and eucaryotic cells is inhibited by cucurbitacins *in vitro* and that many of the compounds are cytotoxic. Bartalis and Halaweish (2005) have shown that lipophilicity (as defined by reversed-phase high-performance liquid chromatography hydrophobicity index) increases the basal toxicity of cucurbitacins on HepG2 (human hepatocellular carcinoma) cells.

The observation by Duncan and Duncan (1997) that proliferating primary and immortalised endothelial cells are significantly more sensitive than confluent endothelial cells to low concentrations (50 nM for 24 h) of cucurbitacin E (LD₅₀-ies for log-phase cells were 12 and 13 nM, respectively, vs. 170 nM and 76 nM, respectively for confluent cells), might indicate that growing tissues and cells are more susceptible to cucurbitacins than stationary cells. This speculation is supported by the finding that exposure of primary prostate carcinoma explants and immortalised prostate carcinoma cells to cucurbitacin E leads to a cytotoxic block characterised by inhibition of cell growth, and development of morphologically abnormal, multinucleated cells (Duncan *et al.*, 1996). A prominent effect of the cucurbitacin E exposure was a disruption of the F-actin cytoskeleton, and accumulation of filamentous, polymerised actin concomitantly with disappearance of G-actin. The cytotoxic block could, therefore, be due to a disrupted formation of an actin-derived contractile ring, a structure important for cell division during mitosis. The cytokeratin filaments and the microtubule cytoskeleton were not altered.

Cucurbitacin E has also been shown to have immunomodulatory effects on peripheral human lymphocytes (Attard *et al.*, 2005). Survival of human cancer cells (PC-3 from prostate cancer, and ZR-75-1 from breast cancer) were not influenced by co-cultivation with peripheral human lymphocytes. When the co-culture of cancer cells and peripheral human lymphocytes were supplied cucurbitacin E at concentrations that had marginal effects on each separate cell line, a cytotoxic effect on the cancer cells were evident. The authors concluded that the combined effect of peripheral human lymphocytes and cucurbitacin E on cancer cells indicated that the

cytotoxic T-cell subset was activated and effectively caused cancer cell death (Attard et al., 2005)

One of the reasons for cucurbitacins being cytotoxic *in vitro* could be that the compounds influence cell adhesion to culture vessels. Very low concentrations of cucurbitacin E and cucurbitacin B, less than 1 μM , was shown to inhibit adhesion of transformed B cells (Musza et al., 1994). The C25-deacetylated forms of cucurbitacins I and D were both about fivefold less potent, and the C23 - C24 dihydrogenated compounds inactive at concentrations up to 50 μM . Thus, an acetyl group at C25 is important and a double bond between C-atoms 23 and 24 vital for cell adhesion. The double bond between C5 and C6, however, seems not to be required.

Table 4. Results of in vitro studies on cytotoxicity of cucurbitacins.

Compound	Test system	Result	Reference
Cucurbitacin B from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	High activity	Konopa <i>et al.</i> , 1974b
Cucurbitacin B from roots of <i>Marah oreganus</i>	Growth of human carcinoma of the nasopharynx KB cells	Inhibition of growth (ID_{50}) at 5.3×10^{-10} - 2.5×10^{-6} $\mu\text{g/ml}$	Kupchan <i>et al.</i> , 1967
Cucurbitacin B from fruits of <i>Cucurbita andreana</i>	Growth of HCT-116 (colon), MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS) cancer cells.	Inhibition by 82-96% at a concentration of 0.4 μM .	Jayaprakasam <i>et al.</i> , 2003
Isocucurbitacin B from roots of <i>Marah oreganus</i>	Growth of human carcinoma of the nasopharynx KB cells	Inhibition of growth (ID_{50}) at 0.4 $\mu\text{g/ml}$	Kupchan <i>et al.</i> , 1967
Dihydrocucurbitacin B from roots of <i>Marah oreganus</i>	Growth of human carcinoma of the nasopharynx KB cells	Inhibition of growth (ID_{50}) at 1.7×10^{-3} - 2.6×10^{-2} $\mu\text{g/ml}$	Kupchan <i>et al.</i> , 1967
Cucurbitacin B from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC_{50}) at 27.7 μM	Bartalis and Hala-weish, 2005
Cucurbitacin D from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	High activity	Konopa <i>et al.</i> , 1974b
Cucurbitacin D	Growth of 9 different bacterial strains of the genera <i>Xenorhabdus</i> and <i>Photorhabdus</i>	Growth of 4 strains were inhibited, there was no effect in 4 strains and stimulated growth in one	Barbercheck and Wang, 1996
Cucurbitacin D from fruits of <i>Cucurbita andreana</i>	Growth of HCT-116 (colon), MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS) cancer cells.	Inhibition by 25-80% at a concentration of 0.4 μM .	Jayaprakasam <i>et al.</i> , 2003
Cucurbitacin D from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC_{50}) at 77.3 μM	Bartalis and Hala-weish, 2005
Isocucurbitacin D from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC_{50}) at 80.3 μM	Bartalis and Hala-weish, 2005
Cucurbitacin E from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	High activity	Konopa <i>et al.</i> , 1974b
Cucurbitacin E from roots of <i>Marah oreganus</i>	Growth of human carcinoma of the nasopharynx KB cells	Inhibition of growth at (ID_{50}) 5.8×10^{-9} - 4.5×10^{-7} $\mu\text{g/ml}$	Kupchan <i>et al.</i> , 1967
Cucurbitacin E	Growth of primary prostate carcinoma explants and immortalised prostate carcinoma cells	Inhibition of growth at 50 nM for 48 h	Duncan <i>et al.</i> , 1996

Compound	Test system	Result	Reference
Cucurbitacin E from fruits of <i>Cucurbita andreana</i>	Growth of HCT-116 (colon), MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS) cancer cells.	Inhibition by 27-77% at a concentration of 0.4 μ M	Jayaprakasam <i>et al.</i> , 2003
Cucurbitacin E from fruits of <i>Ecballium elaterium</i>	Growth of PC-3 (prostate) and ZR-75-1 (breast) cancer cells in the presence of peripheral human lymphocytes	20 μ M promoted T-cell dependent cytotoxicity	Attard <i>et al.</i> , 2005
Cucurbitacin E from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC ₅₀) at 15.3 μ M	Bartalis and Hala-weish, 2005
Cucurbitacin E β -D-glucopyranose from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC ₅₀) at 226.7 μ M	Bartalis and Hala-weish, 2005
Cucurbitacin F from fruits of <i>Cucurbita andreana</i>	Growth KB cells (IC ₅₀ =0.074 μ g/ml) and P-388 murine leukemia cells (IC ₅₀ =0.04 μ g/ml) (Fang <i>et al.</i> , 1984).	Cucurbitacin F (with a double bond in the side chain) had a strong cytotoxic activity with IC ₅₀ values of 0.074 and 0.04 μ g/ml, respectively.	Fang <i>et al.</i> , 1984
23,24-Dihydrocucurbitacin F and 3 β -(β -D-glucosyloxy)-16 α ,23 α -epoxycucurbita-5,24-dien-11one from <i>Kageneckia oblonga</i>	Growth of P-388 murine leukemia cells, A-549 human lung carcinoma and HT-29 colon carcinoma cells.	Very weakly cytotoxicity was observed (IC ₅₀ =5 μ g/ml).	Delporte <i>et al.</i> , 2002
Cucurbitacin I from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	High activity	Konopa <i>et al.</i> , 1974b
Cucurbitacin I from fruits of <i>Cucurbita andreana</i>	Growth of HCT-116 (colon), MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS) cancer cells.	Inhibition by 2-65% at a concentration of 0.4 μ M.	Jayaprakasam <i>et al.</i> , 2003
Cucurbitacin I from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC ₅₀) at 15.8 μ M	Bartalis and Hala-weish, 2005
Cucurbitacin I β -D-glucopyranose from fruits of <i>Cucurbita texana</i> and <i>Citrullus lanatus</i>	Growth of human hepatocellular carcinoma HepG2 cells	Cytotoxicity (IC ₅₀) at 390.0 μ M	Bartalis and Hala-weish, 2005
Cucurbitacin J from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	Very slight activity	Konopa <i>et al.</i> , 1974b
Cucurbitacin K from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	Very slight activity	Konopa <i>et al.</i> , 1974b
Cucurbitacin L from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	Moderate activity	Konopa <i>et al.</i> , 1974b
Tetrahydro-cucurbitacin I from roots of <i>Bryonia alba</i>	Reduction in protein synthesis in two human carcinoma cell lines (KB and HeLa)	Moderate activity	Konopa <i>et al.</i> , 1974b
25-Acetoxydihydrocucurbitacin F	Gene expression of cultured <i>Drosophila melanogaster</i> cells	Cytotoxicity at around the 1 mM level	Dinan <i>et al.</i> , 1997
3 β -(β -D-Glucosyloxy)-16 α ,23 α -epoxycucurbita-5,24-dien-11one from aerial parts of <i>Kageneckia oblonga</i>	Growth of P-388 (mouse lymphoid), A-549 (human lung), and HT-29 (human colon) cancer cells.	No cytotoxicity (inhibition of growth required more than 10 μ g/kg)	Muñoz <i>et al.</i> , 2000
Extract from twigs of <i>Gonystylus keithii</i> (containing cucurbitacin D)	Growth of a battery of cell lines used by the National Cancer Institute	More or less pronounced cytotoxicity in the different cell lines	Fuller <i>et al.</i> , 1994

Another effect reported after exposure of primary prostate carcinoma explants and immortalised prostate carcinoma cells to cucurbitacin E (50 nM, 48 h) is the development of membrane blebs (Duncan *et al.*, 1996). Blisters have also been observed to be formed in human lymphocytes exposed to cucurbitacin D (0.1 µg/10⁶ cells) for 1 h at 37°C. Lymphocytes incubated with 0.1–5 µg cucurbitacin D/million cells remained viable, although the compound was absorbed into the cells.

The efficiency of blister formation differed between different types of lymphocytes. Lymphocytes obtained from patients with chronic lymphatic leukaemia or lymphosarcomas were at least 5 times more sensitive to cucurbitacin D-induced blister formation than normal human lymphocytes. It is not known whether the blebs and blisters induced *in vitro* in cucurbitacin-exposed cells and observed in intoxicated animals, are due to an interference with the cell cytoskeleton.

In addition to cucurbitacins being active on proliferating cells, there are indications of cucurbitacins interfering with various cellular receptors. By studying how cucurbitacins competed with the binding of ³H-cortisol to the isolated glucocorticoid receptor of HeLa cells and the same receptor in intact cells at two different temperatures, and correlating the inhibiting activity with cucurbitacin-induced cytotoxicity, Witkowski and Konopa (1981) drew the conclusion that cucurbitacins are metabolised under physiological conditions. They also came to the conclusion that cucurbitacins bind to glucocorticoid receptors and result in cytotoxic action.

Interestingly cucurbitacin R diglycoside (DCR), which is one of the active principles of *Bryonia alba* roots, may influence the production of corticosteroids and the biosynthesis of eicosanoids in adrenal cortex, isolated adrenocortical cells, and leukocytes. The activity has been noted both under stress and under stress-free conditions, and to occur both *in vivo* and *in vitro*. The cucurbitacin R diglycoside prevents manifestation of stress-induced alterations of eicosanoids in blood and moderately stimulates the adrenal cortex to adapt the organism to stress, because a moderate increase in corticosteroid secretion protects the defence system of organisms from becoming hyperactive (Panossian *et al.*, 1999).

Cucurbitacins B and D (from *Iberis umbellata*) and various cucurbitane-like compounds (from *Hemsleya carnosiflora*), including dihydrocucurbitacin F and dihydrocucurbitacin F acetylated at C₂₅, have been identified as antagonists to insect steroid hormones acting at the ecdysteroid (regulation of the pupation) receptor in *Drosophila melanogaster* cells cultured *in vitro* (Dinan *et al.*, 1997a, 1997b). A 50% inhibition of hormone binding to the ecdysteroid receptor was obtained in the presence of 1.5 µM cucurbitacin B, 10 µM cucurbitacin D, and around 100 µM dihydrocucurbitacin F or 25-acetoxydihydrocucurbitacin F. Important chemical structural features for antagonistic activity on the receptor were the presence of a double bond between carbon atoms 23 and 24, a keto function at C₂₂, and a sufficiently long side chain (Dinan *et al.* 1997a).

Cucurbitacin E and I (300 mg/l) and juice from “squirting cucumber” fruits (*Ecballium elaterium*) (67 ml/l) have been shown to inhibit laccase formation (an important function of pathogenic fungi) in the mycelium of the pathogenic fungus *Botrytis cinerea* (Gonen *et al.*, 1996). This observation could indicate a role for cucurbitacins in protection of the plant against microfungi and other attacking organisms. In agreement with such a hypothesis, cucurbitacin C, which may be found in back-mutated cucumbers, inhibits the growth of *Phytophthora cactorium* at a concentration of 10 mg/ml (Nes and Patterson, 1981). Furthermore, in the plant *Iberis amara*, cucurbitacins E and I acts as feeding inhibitors for the flea beetle *Phyllotreta nemorum* (Kvist Nielsen *et al.*, 1977). Cucurbitacin E inhibits cell adhesion through a mechanism involving disruption of the cytoskeleton (Musza *et al.*, 1994).

In addition to giving rise to cytotoxicity and being active through receptor interaction, isolated cucurbitacins or extracts containing cucurbitacins, have been shown to have other biological effects *in vitro*. For example, some of the compounds have been shown to inhibit haemolysis *in vitro* (Huang *et al.*, 1998), nitrite production in mouse macrophages (Delporte *et al.*, 2002; Park *et al.*, 2004), and protease (elastase) release in stimulated human neutrophils (Delporte *et al.*, 2002).

On the other hand, several cucurbitacins had no or very weak anti-inflammatory and anti-oxidant effect in human tumour cells cultured *in vitro* (Jayaprakasam *et al.*, 2003).

8.2 In vivo toxicity

Cucurbitacins give rise to pronounced acute toxicity. This has been documented in experimental animals and man.

8.2.1 Acute toxicity in experimental animals

A series of cucurbitacins, some of their β -glycosides, and their 2,16-diacetylated and 23,24-dihydrogenated derivatives have been studied to establish the relationship between chemical structure and toxicity. The data given in table 5 are the LD₅₀ values in mice (strain not given) after oral administration of various cucurbitacins and their glycosides (Le Men *et al.* 1969).

It was demonstrated that the most toxic cucurbitacins (D and I) have an unsaturated side chain (Δ_{23}) and a free hydroxyl group at C₂₅. The most purgative ones (B, C, and E) also have a double bond in the side chain (Δ_{23}), but an acetyl group at C₂₅ (Le Men *et al.*, 1969).

Not unexpectedly, cucurbitacins are much more toxic (LD₅₀) after intra-peritoneal than after oral administration, mice being a little more sensitive than rats to cucurbitacin A and cucurbitacin C (Table 6) but a little

less sensitive to cucurbitacin D (Table 7). It should be emphasised that the data in Table 6 are based on studies using only 3 dose levels with no more than 5 males and 2 females per dose. The LD₅₀ values were estimated according to the formula of Kärber (David and Valance, 1955).

The data in Table 6 were partly confirmed by Tessier and Paris (1978), who isolated and characterised cucurbitacin A, 23, 24-dihydrocucurbitacin A, cucurbitacin E and cucurbitacin L, and tested the toxicity of these compounds by intraperitoneal administration in mice. A single administration of each compound, all at a dose of 10 mg/kg body weight and above caused purgation and disturbed respiration leading to death within 12 to 24 hours.

Table 5. LD₅₀-values (mg/kg body weight) in mice after oral administration (gavage) of cucurbitacin derivatives.

Cucurbitacin derivative	Toxicity – LD ₅₀
Cucurbitacin B	LD ₁₀ : 5
Cucurbitacin C	100
Cucurbitacin D	5
Cucurbitacin E	340
β-Glycoside of cucurbitacin E	40
Cucurbitacin I	5
β-Glycoside of cucurbitacin I	650

Table 6. LD₅₀* (mg/kg body weight) of cucurbitacins after intra-peritoneal administration.

Cucurbitacin	Mouse (albino)	Rat (albino)
Cucurbitacin A	1.2	2
Cucurbitacin B	1	(not tested)
Cucurbitacin C	0.8	> 4

* estimated according to the formula of Kärber (David and Vallance, 1955)

Cucurbitacin D is the most studied cucurbitacin. Acute toxicity studies with the compound have been performed in the mouse, rat, cat and dog (Table 7). The symptoms of toxicity varied between species, but included periods of tachypnoea separated by intervals with normal respiration, diarrhoea, and adynamia, followed by severe prostration, coma and death. In cats and dogs vomiting was pronounced (Edery *et al.*, 1961). The latency period in cats and dogs was as short as 45 minutes, whereas it was more than 6 and 10 hours in mice and rats, respectively, even after exposure to high doses of cucurbitacins (Edery *et al.*, 1961). Pathological studies of animals killed by high doses of cucurbitacin D revealed congestion of the intestine and pancreas, and sometimes also of the kidneys and the liver. The lungs were strongly oedematous due to large amounts of fluid. In some cases appreciable amounts of fluid were found also in the thoracic and abdominal cavities (Edery *et al.*, 1961).

A minimum lethal dose of intravenously injected cucurbitacin A and B for rabbits has been determined to 0.7 and 0.5 mg/kg body weight,

respectively (Enslin, 1954). Following intravenous administration of an anaesthetised cat with a combination of up to 0.32 mg of cucurbitacin A and of up to 0.32 mg cucurbitacin B per kg body weight, no pronounced immediate effects were recorded with respect to respiration, blood pressure and survival. However, after administration of a combination of 0.7 mg cucurbitacin A and 0.3 mg cucurbitacin B per kg body weight the cat died in pulmonary oedema (David and Vallance, 1955).

Table 7. LD₅₀ (mg/kg body weight) of cucurbitacin D in various species and after different routes of administration. Routes of administration: i.v. = intravenously; s.c. = subcutaneously; i.p. = intra-peritoneally.

Animal	Ederly <i>et al.</i> , 1961				Le Men <i>et al.</i> , 1969
	Route				Route
	i.v.	s.c.	Oral	i.p.	Oral
Mouse	0.96	4.6	6.3	1.75	5
Rat		3.4	8.2	1.3	
Cat	0.9 ⁽¹⁾				
Dog	1.0 ⁽¹⁾				

Approximate figure obtained from twelve animals.

An assay for anti-inflammatory activity was performed on male mice with fractionated fresh fruit juice of *Ecballium elaterium*. A number of fractions of the water-soluble extract of the fruit juice increased vascular permeability. The most effective fraction was shown to contain cucurbitacin B. The fruit juice containing as much as 24.8 g cucurbitacin B per litre (Sezik, 1997).

Experimental studies on rabbits with specimens of a bitter-tasting vegetable marrow of the smooth-skinned maranc, that had poisoned a young boy, showed in experimental studies that animals became restless, got a very pronounced accelerated pulse and accelerated respiration. The pulse became progressively weaker and the respiration progressively more laboured until, within two to three hours after having been dosed with the maranc, the animals died in convulsions due to asphyxia. In acute cases restlessness, nervousness, dyspnoea, anorexia, and profuse diarrhoea appeared, with an initially accelerated and strong pulse that became weaker over time. Post-mortem studies revealed general cyanosis, pronounced dilatation of both heart ventricles and atria (distended with coagulated blood), slight oedema and pronounced hyperaemia of the lungs, and severe hyperaemia of the gastric mucosa (Steyn, 1950).

8.2.2 Subacute/subchronic toxicity in experimental animals

Ten groups of 8 weaning, male, Swiss Webster mice were fed a basal diet (controls), or a basal diet containing 1, 10 or 20 % of Straightneck squash, Blackjack squash, or *Cucurbita texana* fruit for 10 weeks. The animals were allowed to consume the different feeds *ad libitum*. The fruits were added to the feed as dried powder (peeled, de-seeded, and

freeze-dried fruits, grounded to a fine powder) replacing sucrose. Both squash cultivars were free of cucurbitacins whereas the *Cucurbita texana* fruit contained 3.56 g cucurbitacin E glycoside and 1.39 g cucurbitacin I per kg fresh fruit, i.e. the feed with *Cucurbita texana* contained around 0.05, 0.5 and 1 g total cucurbitacins per kg feed, respectively. All of the animals in the 10 % and 20 % *Cucurbita texana* groups died within 3 to 6 days. Three of the eight animals in the 1 % *Cucurbita texana* group died during the course of the 10-week study. The surviving animals of this group showed reduced body weight gain and severe diarrhoea both when compared to mice fed the basal diet and when compared to mice fed the two non-bitter squash cultivars (any of the concentrations). Haematological investigations of the surviving mice fed 1 % *Cucurbita texana* showed reduced haemoglobin levels, haematocrit and red blood cell count. Normal feed consumption, growth, and haematological profile were observed in animals fed all three levels of Straightneck or Blackjack squash (Stoewsand *et al.*, 1985).

A series of studies have been performed with purified cucurbitacin D on mice, rats, cats, dogs, rabbits and monkeys by Edery *et al.* (1961). An increase in the capillary permeability appeared to be the main toxicological effect produced by cucurbitacin D. No liberation of histamine could be demonstrated either in intact animals or in a perfused paw after cucurbitacin D treatment. The fall in systemic as well as portal blood pressure seemed to be due mainly to the reduction in circulating fluid caused by the increased capillary permeability. The fluid appeared to accumulate in the viscera and in the cavities, which was ascertained by lung oedema and ascites observed in autopsied animals. The action of cucurbitacin D on the heart seemed to be of secondary importance to the blood pressure fall. Bradycardia was observed in intact animals as well as in isolated heart treated with cucurbitacin D, pointing to the conduction being impaired. The effect on the respiratory system appeared to be of a peripheral nature, as the excitability of the respiratory centre remained unaltered even after large doses of cucurbitacin D. The deepening of the respiratory movements was apparently due to an increased bronchial tone, which could be caused by an accumulation of fluid around the alveoli. Cucurbitacin D irritate the intestinal mucosa strongly and increased intestinal motility, but had no action on isolated intestine. It seems less likely that cucurbitacin D possess an important central activity. Synergism was, however, observed between cucurbitacin D and sodium thiopentone and could be explained by a facilitation of the penetration of the narcotic into the brain cells. Numerous pathological changes were found in cumulative dosing tests. The liver showed hepatomegaly, perihepatitis and fatty degenerations. Perisplenitis and inflammation of the gastro-splenic ligament were found along with interstitial nephritis and congested lungs, showing patches of compensatory emphysema (Edery *et al.*, 1961).

8.2.3 Subacute/subchronic toxicity in domestic animals

Barri, Elewad and co-workers have performed several feeding studies with leaves and fruit from the two cucurbitaceous plants the colocynth (*Citrullus colocynthis*) and the bottle or calabash gourd (*Lagenaria siceraria*). Although the cucurbitacin content of these experimental materials is unknown, the results is briefly described below.

Among the Sudanese plants credited with medicinal properties two cucurbits have been tested for acute or sub-acute toxicity – the colocynth (*Citrullus colocynthis*) – fruit and leaves – and the bottle gourd or calabash gourd (*Lagenaria siceraria*) – leaves, fruits and seeds.

Very high toxicity of *Citrullus colocynthis* was shown in studies on Nubian goats, sebu calves, and dessert sheep. Daily doses of 1g/kg body weight of fresh fruit of *C. colocynthis*, dried seeds and dry fruit pulp resulted in death within 10 days of goats fed with stomach tube. Three goats given 0.25 g dried colocynth leaves per kg feed died on days 7, 8, 9 of the study, respectively. Calves were fed similar amounts of (1 or 10 g) colocynth fruits/kg body weight. The three calves given 10 g fruit/kg body weight died after 18 hours, 2 days and 15 days, respectively. In both goats and calves, the first clinical sign was diarrhoea. Other signs were inappetence, reduced water intake, uneasiness, dyspnoea, recumbency and lateral bending of the head and neck. Dehydration, loss of condition, sunken eyes and anaemia were prominent signs in goats and calves given the low dose. Post mortem findings in goats given fruits or leaves were haemorrhages in the endocardium, kidney, spleen and gall bladder, congestion of the lungs and liver, enteritis and pulmonary emphysema. Oedema of the gall bladder wall and focal necrosis of the liver were prominent in goats given 0.5 and 5 g/kg body weight/day. Calves given the high dose showed the same signs as the goats but haemorrhage of the endocardium and the gall bladder wall was more marked. There was severe hepatic necrosis and gelatinisation of the renal pelvis and epicardial fat in low dose calves (Barri *et al.*, 1983).

Groups of two male desert sheep were given daily drenches of 1, 5, or 10 g minced fresh colocynth fruit (suspended in water)/kg body weight or daily drenches of either 0.25 or 10 g minced fresh colocynth leaves/kg body weight. Two desert sheep received normal feed. The high doses were acutely toxic and lethal. The signs of toxicity in the groups receiving the low doses included diarrhoea, difficulty in respiration, loss of condition, weakness of hind limbs, recumbency and lateral bending of the neck. Symptoms developed between days 5 and 8. The animals died between day 9 and 25. Findings in most animals at necropsy included catarrhal enteritis of the intestine, haemorrhage, fatty changes and congestion of the kidneys and fatty change, haemorrhage and congestion of the liver. Cholecystitis, congestion and haemorrhage of the gall bladder were found in all groups except the one given the highest dose. Congestion, haemorrhage and emphysema of the lungs and haemorrhage and congestion of the

heart were present in all groups. Hydroperitoneum, hydropericardium and hydrothorax affecting the serous cavities were found in the group given 1 g minced fruits/kg body weight/day. Histopathology revealed mainly fatty cytoplasmic vacuolisation in centrilobular hepatocytes, cells of the renal tubules and cardiac muscle fibre cells. Congestion of hepatic sinusoids, renal and cardiac vessels, and pulmonary alveolar capillaries were evident. The only consistent feature in serum was a decrease in total serum protein, which could be secondary to hepatic dysfunction. The haematological studies revealed a concomitant increase in packed cell volume (haematocrit). Haemoglobin values and red blood cell counts pointing towards haemoconcentration (Elawad *et al.*, 1984).

Goats, two animals per treatment, were gavaged with *Lagenaria siceraria*: 1 or 5 g fresh fruit, 5 g dried leaves (powder), 1 or 5 g dried seeds (powder), or 1 g dry fruit pulp/kg body weight/day. The treatment resulted in pronounced toxicity. Most of the goats showed reduced appetite, dullness, diarrhoea, dyspnoea, recumbency and lateral deviation of the neck, and died or were killed within the first 8 days. The rest of the animals showed less marked signs of toxicity and did not die. They were killed between day 10 and 30 after the beginning of the feeding experiment. The post-mortem studies revealed similar lesions in all goats receiving the higher dose of *Lagenaria* fruits, leaves and seeds. Among the lesions were congestion and/or haemorrhage of the liver, lungs, kidneys and abomasums, catarrhal enteritis, pulmonary emphysema and hepatic fatty changes. Goats receiving the lower dose of fruits and seeds showed flaccid hearts with serious atrophy and presence of a straw-coloured fluid in the pericardial cavity. No control animals died during the studies or showed pathological changes at autopsy (Barri *et al.*, 1983).

Seven days old Bovans-type chicks were given a feed with 2% or 10% seeds of *Citrullus colocynthis* for 6 weeks. During the experiment, the average body weight and efficiency of food utilisation were significantly reduced in the group that was fed 10 % seeds. Studies on the blood showed significantly increased activities of lactate dehydrogenase, aspartate aminotransferase and creatinine kinase and increased concentrations of total lipid and zinc in blood. Lesions observed in the intestine, liver, kidneys and other tissues were reversed 4 weeks after removal of the *Citrullus colocynthis* seeds from the experimental diet (Bakhiet and Adam, 1995).

The common feature of all toxic effects reported in experimental and domestic animals exposed to cucurbitacins isolated and purified from plants, as well as cucurbitacin containing plant material is a violent irritation of the intestinal mucosa, and after prolonged exposure appearance of hepatic fatty changes, catarrhal enteritis, pulmonary emphysema and necrosis of the cells of the renal tubuli.

8.2.4 Subchronic anti-tumourigenicity studies in experimental animals

No data were available on chronic toxicity of any cucurbitacin or cucurbitacin glycoside in healthy animals or tissues. Some of the compounds have, however, been tested for chemotherapeutic activity against initiated or tumour cells.

Administration (no information available about route) of 0.8–1.6 mg cucurbitacin B/kg body weight inhibited tumour development in rats implanted with Walker carcinosarcoma 256 and mice implanted with Lewis lung carcinoma. However, as the margin between the active and the toxic dose was small, the compound was declared unpromising as a therapeutic agent. Cucurbitacin E had a very low activity against Walker carcinosarcoma 256 in rats (Kupchan *et al.*, 1967).

In a similar study, Konopa *et al.* (1974b) injected cucurbitacin B, D, E, I, J, K, L and tetrahydrocucurbitacin I into mice implanted with solid Crocker sarcoma 180 cells or injected Ehrlich ascites cells, starting 24 hours after implantation/injection of cells and repeated every 24 hours for 6–10 days. The amounts of cucurbitacins administered were approximately equal to the maximum tolerated dose determined in the study (0.4–0.8 mg/kg body weight for cucurbitacins B, D, and I, and 4 mg/kg for cucurbitacin E). All tested cucurbitacins were cytotoxic to the KB and HeLa indicator cells *in vitro*. Although cytotoxic *in vitro*, cucurbitacins J, K, L and tetrahydro-cucurbitacin I did not inhibit the growth of the implanted tumour cells in the *in vivo* studies.

A number of cucurbitacins (21 cucurbitane triterpenoids isolated from the rhizomes of two Chinese plants, *Hemsleya panacis-scandens* and *H. carnosiflora*, or from the twigs and leaves of an American plant, *Cowania mexicana*, as well as various 24,29-*nor*-cucurbitacin glucosides isolated from the roots of a Chinese cucurbitaceous plant, *Cayaponia tayuya*) have been screened for influencing the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen activation in lymphoblastoid cells (Konoshima *et al.*, 1993, 1994, 1995). Three of the 21 cucurbitane triterpenoid compounds and 3 of the 24,29-*nor*-cucurbitacin glucosides inhibited activation of the tumour cells *in vitro*. Among the active inhibitors were cucurbitacin F, 15-oxo-cucurbitacin F and the 23,24-dihydrogen compounds of these cucurbitacins. Some of the active substances were subsequently tested in an *in vivo* two-stage mouse skin promotion test where papillomas were initiated with DMBA (7,12-dimethylbenz[*a*]anthracene) and promoted by TPA. After treatment for 20 weeks with the various cucurbitacins (85 nM), pronounced anti tumour-promoting activity was obtained with 23,24-dihydrocucurbitacin F, 23,24-dihydrocucurbitacin F glycoside, and cayaponoside B and C₂.

STAT3 is a protein involved in signal transduction. It is often found tyrosine phosphorylated and constitutively activated in many human cancer types. Among nearly 2000 compounds in the National Cancer Institute compound library, the most effective inhibitor of STAT3 activation in

several human cancer cell lines is cucurbitacin I (Blaskovich et al., 2003). Cucurbitacin I specifically suppress the phosphotyrosine level of STAT3 without affecting the protein level. The cucurbitacin also disrupt STAT3 DNA-binding activity and STAT3-mediated gene expression, thereby affecting STAT3-dependent signal transduction. It induced apoptosis in cells constitutively expressing activated tyrosine-phosphorylated STAT3. However, the compound inhibited cellular proliferation independently of STAT3 activation status and inhibited growth of transplanted tumours in immuno-deficient and immuno-competent mice as long as the transplanted tumours had constitutively activated STAT3. As a consequence, life span was increased. However, the injections with cucurbitacin I caused oedema at the site of injection.

9. Human data

The extreme bitterness of cucurbitacins should hinder humans from being exposed to substantial quantities of the compounds. Nevertheless, some poisonings have been reported after consumption of cucurbitaceous food plants. The South African Steyn (1932) reports that he first was made aware of toxic bitter-tasting vegetable marrow of the smooth-skinned maranc (*Cucumis* sp.) in 1932. A young boy poisoned by the bitter vegetable marrow showed digestive disturbances (vomiting, diarrhoea), prostration, and very severe dyspnoea. When tested on rabbits the vegetable marrow proved to be extremely toxic (Steyn, 1935, 1936). Within three hours after administration, quantities down to 7 g, killed full-grown animals. In the eighteen years that followed Steyn received specimens of vegetable marrow, little gems, watermelon and golden custard squash which had bitter taste or which had resulted in intoxication when consumed (Steyn, 1950). As little as 2.0 g of either fresh or cooked specimen per kilogram body-weight often proved to be fatal to rabbits.

In 1982 the Australian Department of Health reported 22 cases of intoxication in Queensland after consumption of zucchini squash. Two different cultivars were found to be responsible for the episodes. Trace backs were to the Blackjack cultivar in 21 cases and to the Castleverde cultivar in one case.

Symptoms of severe cramps, persistent diarrhoea, vomiting, headaches and collapse have been reported to occur within 1 to 2 hours after ingestion of as little as about 3 g of zucchini in the United States (Rymal *et al.*, 1984). Rymal and co-workers (1984) obtained fresh, bitter yellow straightneck squash fruits from farms in Alabama that had produced fruits leading to intoxication. The fruits were found to contain 3100 mg cucurbitacin E/kg fresh weight. Frozen raw zucchini fruit pieces of the Castleverde cultivar, being responsible for another intoxication, were also analysed for cucurbitacin E and found to contain 7200 mg/kg of flesh at the stem end, and 2700 mg/kg in the central portion of the fruit.

Gastrointestinal symptoms have also been reported in a Japanese person consuming the bottle gourd, which contained cucurbitacin D (Tamura *et al.*, 1983). Poisoning after consumption of a bitter type of edible gourd has been reported also in China (Anonymous, 1983). Furthermore, it should be mentioned that regular consumption of large quantities of apparently non-bitter squash juice have been reported to cause failure to thrive (Hope and Foote, 1995). In this case, it is not known which squash constituent is responsible for the observed effect.

No data on poisoning have been available from the Nordic countries before the middle of the 1990'ies. As mentioned in the introduction, re-

ports on poisoning from bitter zucchini occurred in Denmark 1995 and 1996 (Pilegaard and Søborg, 1995). Cases of squash intoxication have occurred also in Sweden in 1996 and 2004 (Andersson, personal communication). Unfortunately, no material from the squash “meals” resulting in intoxication was saved for future analytical work to identify and quantify the “bitter (and toxic) principles”.

Fruit juice of *Ecballium elaterium* has been used since antiquity in folk medicine in the Mediterranean basin as a potent cathartic, analgesic, and anti-inflammatory agent. A clinical study on 49 voluntary patients with sinusitis revealed the fruit juice to have healing activity. The active anti-inflammatory principle was isolated as 2.48% of the fruit juice and identified as cucurbitacin B (Sezik, 1997). Raikhlin-Eisenkraft and Bentur (2000) report on a series of 13 patients using the juice as medicine. In 3 patients, exposure was intranasal for the treatment of sinusitis or liver cirrhosis. In 3 other cases, children ingested the fruit unwittingly. In 6 patients, exposure was ocular and – in one – dermal. Within minutes of exposure, the patients exhibited irritation of the mucous membranes. The severity varied with the treatment, depending on the route of exposure. The effects were manifested as oedema of the pharynx, dyspnoea, drooling, dysphagia, vomiting, conjunctivitis, corneal oedema, and erosion. Recovery began several hours after administration of oxygen, steroids, antihistamines, and beta-2-agonists. It was concluded that exposure to the undiluted fruit juice of the “squirting cucumber” may cause irritation of mucous membranes, supposedly of inflammatory nature. The toddler receiving dermal exposure in the study of Raikhlin-Eisenkraft and Bentur (2000) referred to above, remained asymptomatic. Absence of irritating properties following dermal exposure (forearm) have been reported also after exposure to purified cucurbitacin D (Edery *et al.*, 1961).

10. Conclusions

During many years of careful selection of which type of plants to cultivate, and a century of plant breeding, the cultivated cucurbitaceous food plants have lost their wild type capacity to produce cucurbitacins, which are toxic and immensely bitter compounds. The assumed precursor for the cucurbitacins, cucurbita-5,24-dienol, have, however, been identified in some of these food plants. The reason for the absence or suppressed levels of cucurbitacins in cultivated plants is not known, but it has been suggested to be the result of a mutation (in a structural gene or a suppressor gene). If this assumption is correct, back mutations occurring rarely would allow a spontaneous reappearance of plants containing cucurbitacins. These plants would produce bitter and toxic fruits. At least some of the offspring of such plants will continue to produce cucurbitacins.

Very few data are available on human intoxications after consumption of cucurbitaceous food plants. It is known, however, that irritation of mucous membranes manifested as oedema of the pharynx, dyspnoea, drooling, dysphagia, and vomiting, occur within minutes of oral exposure to preparations containing cucurbitacins. In general the cucurbitacin content of the ingested material that have led to intoxications is unknown. In 1982 the Australian Department of Health reported more than 20 cases of intoxication in Queensland after consumption of zucchini squash. The symptoms included stomach cramps, diarrhoea, vomiting and headaches. Symptoms of severe cramps, persistent diarrhoea and collapse have been reported within 1 to 2 hours after a single ingestion of as little as about 3 g zucchini. In the middle of the 1990's, intoxications after squash consumption was reported also in Denmark and Sweden.

Cucurbitacins have been detected in about 100 of the 900 known species in the *Cucurbitaceae* family. The highest cucurbitacin concentrations are normally found in roots, which are never consumed as food. However, roots of some species have been, and are still used as traditional medicines. It is the fruits of cucurbitaceous plants that are consumed as food. No plant foods other than from the *Cucurbitaceae* family have ever been reported to contain cucurbitacins.

The most abundant cucurbitacins in cucurbitaceous food plants are cucurbitacin B, (C), D, E, I and L. Usually only a few of these, particularly cucurbitacin E glycoside, has been detected in bitter fruits of plants accidentally back-mutated to bitter forms. It is still not known whether aglycones are normally present in the intact cells or whether they are formed by the action of β -glucosidase being activated by cell destruction during the preanalytical steps of sample preparation. β -2-Monoglycosides of cucurbitacins are very common. The sugar moiety in the

cucurbitacin glycoside may be D-glucose or L-rhamnose. A special series of cucurbitacins – momordicosides – found in *Momordica charantia*, which is very rarely used as food, differ so much from cucurbitacins in other cucurbitaceous plants that they have not been discussed further in this report.

Cucumber, squash and other pumpkins, calabash, melons, and watermelons, have all been reported to sometime produce bitter cucurbitacin-containing fruits. Other cultivated cucurbitaceous plants eaten elsewhere in the world – chayote fruit and snake gourds – have never been found with bitter fruits. However, seedlings of both these plants are known to contain cucurbitacins. Thus, they do have the genetic constitution required for synthesis of such compounds. The "Bitter melon" fruit used in oriental cooking is harvested and consumed when still white-fleshed and immature. At this stage the fruits are least bitter. The mature Bitter melon fruit tend to split open revealing orange fruit flesh and red seeds and is reported to be toxic.

In the Nordic societies, the rare exposure to cucurbitacins usually comes from consumption of bitter fruit of back-mutated cucurbitaceous food plants. Fruits of these plants are normally non-bitter and totally free of cucurbitacins. Cucurbitacin E glycoside, the least bitter of the studied cucurbitacins is recognised by consumers already when occurring at a level of 2 ppm (2 mg/kg) in squash pulp. Back mutated watermelons and squash have been reported to produce the cucurbitacin E glycoside in concentrations between 930 and 3100 mg/kg. Such fruits are immensely bitter. Thus, due to the very bitter taste, the rare human exposure to cucurbitacins is generally anticipated to be one oral dose of squash/watermelon. One mouthful would correspond to around 20 g fruit containing approximately 18–60 mg cucurbitacins. This cucurbitacin exposure would correspond to a dose between 0.2 and 1.0 mg/kg body weight. Repeated or chronic exposure via food is unlikely due to the random occurrence of cucurbitacin-containing plants and the extremely bitter and disagreeable taste of these plants.

The toxicological information available about cucurbitacins comes from *in vitro* studies and feeding studies in experimental animals and domestic animals, as well as from a few accidental poisonings of humans. Animal feeding studies show that chronic exposure to cucurbitacins or cucurbitacin-containing plant parts results in pronounced toxicity. Severe diarrhoea, reduced haemoglobin levels, haematocrit and red blood cell count were observed in surviving mice fed 0.05 % cucurbitacins for 10 weeks. Sheep and goats fed fresh plant material containing cucurbitacins died with catarrhal enteritis of the intestine and hepatic fatty changes. Chickens fed cucurbitacin-containing plant material developed damage in the intestine and the liver.

Also acute exposures of experimental animals to cucurbitacins lead to toxicity. Although exposure via the oral route is less toxic than exposure

via the subcutaneous, intraperitoneal and intravenous routes, the oral LD₅₀ in mice is as low as around 5 mg/kg body weight for some of the cucurbitacins (cucurbitacin D and I). The main toxicological effect produced by cucurbitacin D appears to be an increase in the capillary permeability. The compound seems to irritate the intestinal mucosa, and strongly increase intestinal motility *in vivo*, but it seems to have no action on isolated intestine. The LD₅₀ of the cucurbitacine E glycoside often detected in the bitter cucurbitaceous food plants is also low, 40 mg/kg body weight. After intraperitoneal administration of 10 mg cucurbitacin A, E or L/kg body weight to mice, purgation and disturbed respiration leading to death occurred within 12 to 24 hours. Histopathological studies of the animals revealed congestion of the intestine and oedematous lungs.

Available information identifies a low likelihood of being exposed to cucurbitacins through normal food consumption. But when the unlikely episode that non-bitter cucurbitaceous food plants back-mutate and regain the ability to produce cucurbitacins occur, the risk for intoxication is very high, particularly if the organoleptic characteristics of the cucurbitacins (bitterness) is masked by other components of the food. The fact that non-bitter cucurbitaceous food plants randomly might regain the ability to produce cucurbitacins through back-mutations, should demand a careful analysis for the production of this type of compounds in genetically modified cucurbitaceous plants, particularly in the fruit.

11. References

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