Jan M.C. GEUNS & Johan G. BUYSE

Proceedings of the first symposium

The Safety of Stevioside

KULeuven, April 16th 2004

Stevia rebaudiana Bertoni
grown in the greenhouse of KULeuven

Euprint ed., Parkbosstraat 3, 3001 Heverlee
info@euprint.be

ISBN 9074253024
EAN 9789074253024
NUR 882 - 893

Titel : Safety of Stevioside, The

D/2004/6045/046

Contents

1. Introduction and Presentation of the"European *Stevia* Research Center".
Jan M.C. Geuns. 5

2. Sensitive HPLC determination of steviol in biological fluids and plant material by fluorescence detection.
Veerle J.Y. Minne, Frans Compernolle, Suzanne Toppet, Jan M.C. Geuns. 9

3. Stevioside as a potentially useful drug in the treatment of type 2 diabetes and metabolic syndrome.
Per Bendix Jeppesen. 25

4. Stevioside metabolism and transport in rats and humans.
Eriko Koyama. 31

5. The metabolism of stevioside by animals: chickens and pigs.
Johan Buyse and Jan M.C. Geuns. 35

6. Bioavailability of stevioside from *Stevia rebaudiana* in human volunteers: preliminary report.
P. Simonetti, C. Gardana, L. Bramati and P.G. Pietta. 51

7. A short-term study of stevioside in healthy subjects.
Elisabeth H.M. Temme, Annelies Vankeirsbilck, Johan Buyse and Jan M.C. Geuns. 63

8. About the safety of stevioside used as a sweetener.
Jan M.C. Geuns, Johan Buyse, Annelies Vankeirsbilck, Liesbeth Temme. 75

9. Review: The safety of stevioside used as a sweetener.
Jan M.C. Geuns. 85

CHAPTER 1

Introduction and Presentation of the "European *Stevia* Research Center"

Jan M.C. GEUNS

Laboratory of Functional Biology, KULeuven,
Kard. Mercierlaan 92, B 3001 Leuven
Tel.: +32-16-321510; Fax: +32-16-321509
e-mail: Jan.Geuns@bio.kuleuven.ac.be

After founding the "European *Stevia* Research Center" (ESC) in 2003 an international symposium was organised on April 16 2004 at the University of Leuven (KULeuven - Belgium) entitled "Safety of stevioside". Three foreign speakers were invited besides Belgian scientists to give an overview of the stevioside research.

Prof. J. Geuns of the Laboratory of Functional Biology, KULeuven, gave an introduction on the European *Stevia* Research Center.
The European *Stevia* Research Center (ESC) was initiated by Prof. Jan M.C. Geuns, Laboratory of Functional Biology, Kasteelpark Arenberg 31, 3001 Leuven (Heverlee) and Prof. Johan Buyse, Laboratory of Physiology and Immunology of Domestic Animals, Kasteelpark Arenberg 30, 3001 Leuven. The ESC is housed at the Laboratory of Functional Biology, Kasteelpark Arenberg 31, 3001 Leuven , Belgium (Tel.: +32-16-321510, Fax: +32-16-321509; e-mail: Jan.Geuns@bio.kuleuven.ac.be).
The main aim of the ESC is the promotion and coordination of all activities focussing on research and health in relation to *Stevia*, stevioside and related compounds. This is

not only in the European Union countries, but also outside the EU and includes developing countries.

In more detail, the ESC has the following aims:

- to be a discussion forum for everyone interested in the research and application of *Stevia*, stevioside and related compounds,
- to prepare a new application for stevioside and related compounds for the EU to lift the ban on stevioside in Europe. This application will be published on the website of the ESC, as required by the EU,
- to develop a "European Quality Label" for stevioside and related compounds, i.e. 95% purity of the mixture of sweeteners, absence of synthetic sweeteners, absence of solvents and absence of steviol. A purity of 95 % is required for the mixture of sweeteners,
- to organise a continuous follow-up of special groups of the population, e.g., diabetics, patients with hypertension, hypotension, etc., after the introduction of stevioside to the European market, as required by the EU guidelines,
- to be a documentation and information center for the consumers,
- to organise workshops and meetings on *Stevia* and stevioside,
- to create a European network to follow-up consumers in the different countries of the EU. In this network a sufficient number of toxicologists and food specialists should be present, speaking different languages of the EU,
- to stimulate research on *Stevia*, stevioside and related compounds,
- to function as an independent source of objective information for the diverse national and European authorities,
- to construct a website providing recent information about *Stevia* and stevioside including answers to FAQs. Links from this website to commercial websites are only possible with the approval of the Executive Board and after full payment of the yearly fees,
- to collect funds for the realisation of its aims.

CHAPTER 2

Sensitive HPLC determination of steviol in biological fluids and plant material by fluorescence detection

Veerle J.Y. MINNE*, Frans COMPERNOLLE**, Suzanne TOPPET**, Jan M.C. GEUNS*

**Lab. Functional Biology, KULeuven, Kasteelpark Arenberg 31, B 3001 Leuven*
Tel.: +32-16-321510; Fax: +32-16-321509;
e-mail: Jan.Geuns@bio.kuleuven.ac.be
*** Laboratory of Organic Synthesis, Celestijnenlaan 200F, B-3001 Leuven.*

ABSTRACT

A simple method is described for the determination of steviol (SV) by reversed-phase high-performance liquid chromatography (RP-HPLC) using dihydro-isosteviol (DHISV) as internal standard (IS). SV and DHISV were derivatised with 4-(bromomethyl)-7-methoxycoumarin in an aprotic solvent (*N,N*-dimethylformamide (DMF) or acetone). Separation of the resulting ester derivatives was achieved on an ODS column (250 × 4.6 mm i.d., 5 μm particle size) at a flow-rate of 1 $mL.min^{-1}$ using acetonitrile-water (80:20 v/v) as the mobile phase. Using fluorescence detection with excitation at 321 nm and emission at 391 nm, a linear relationship was observed for concentrations between 0.5 and 50 μg mL^{-1} of SV and the detection limit was 100 pg. The intra- and interday variations (n = 9) were 0.64 and 0.88%, respectively. The application of the method to beer, urine and faeces samples involved a simple procedure of extraction by diethyl ether and derivatisation in DMF. Plant samples required preparation of an acid fraction containing the SV analyte, derivatization and sample clean-up using small silica

columns made of pipette tips and thin layer chromatography. A sensitive determination of 5.9 µg of SV present in 1 g of dry plant material was done with high precision and accuracy.

KEYWORDS

Stevia rebaudiana (Bertoni) Bertoni, steviol, dihydro-isosteviol, 4-(bromomethyl)-7-methoxy-coumarin, fluorescence detection, biological fluids, plants

INTRODUCTION

Stevia rebaudiana (Bertoni) Bertoni is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil). The main sweet component in the leaves of *Stevia rebaudiana* Bertoni is stevioside. Its content varies between 4 and 20 % of the dry weight of the leaves depending on cultivar and growth conditions (Geuns, 2000). Other compounds present in smaller concentrations are: dulcoside A (*ca.* 0.5 %), steviolbioside (trace), rebaudioside A (*ca.*3 %), B (trace), C (*ca.* 1.5 %), D, E and F (traces). The presence of steviolbioside and rebaudioside B in extracts might be due to artefacts of the extraction procedure (Kennelly *et al.*, 2002; Starratt *et al.*, 2002).

Stevioside is a diterpene glycoside. It is a high intensity sweetener that is about 300 times sweeter than sucrose (0.4% solution). In many countries it is used as a low calorie sweetener in a wide range of food products and beverages. The plant, its extracts, and stevioside have been used for several years as a sweetener in South America, Asia, Japan and China. *Stevia rebaudiana* products are approved for sweetening purposes in Brazil, Korea and Japan. In the United States it has been used as a dietary supplement since 1995 (Kinghorn *et al.*, 2002).

The human body does not take up oral stevioside (Bracht *et al.*, 1985; Koyama *et al.*, 2003a; Yamamoto *et al.*, 1985), and none of the digestive enzymes from the gastrointestinal tract of different animals and man are able to degrade stevioside

CHAPTER 3

Can stevioside be a new useful drug in the treatment of type 2 diabetes and the metabolic syndrome?

Per Bendix JEPPESEN

Ass. Prof., PhD; Aarhus University Hospital, Department of Endocrinology and Metabolism, Tage-Hansens Gade 2, DK-8000 Aarhus C, Denmark.
e-mail: per.bendix.jeppesen@ki.au.dk

Changes in human behaviour and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide. There are two main forms of diabetes, types 1 and 2. The frequency of type 1 diabetes is relatively low compared to type 2 diabetes, which accounts for over 90 % of cases globally. Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion, either of which may predominate, but type 2 diabetes is also a multifactorial disease that shows heterogeneity in many respects [1]. Patients with type 2 diabetes are not dependent on exogenous insulin, but may require it for control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycaemic agents [1]. Our understanding of the disease and related disorders, such as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) is undergoing a radical change, particularly as data suggest that the risk of complications commences many years before the onset of clinical diabetes [2, 3]. Previously, it was regarded as a relatively distinct disease entity, but in reality, type 2 diabetes (and its associated hyperglycaemia or dysglycaemia) is often a manifestation of a much broader underlying disorder [4]. This includes the metabolic syndrome, a cluster of CVD risk factors that, in addition to glucose intolerance includes hyperinsulinaemia, dyslipidaemia, hypertension, visceral obesity,

hypercoagulability and microalbuminuria. The diabetes epidemic is related particularly to type 2 diabetes, and is taking place both in developed and developing counties [5]. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030, especially in developing countries where the incidence of type 2 diabetes is expected to double between 2000 and 2030. Because of limiting economic resources in developing countries, a new and effective low cost treatment for type 2 diabetes is needed [5].

Many medical practitioners with training in pharmacology and pharmacognosy are well aware of the number of modern therapeutic agents that have been derived from tropical forest species. In fact, over 120 pharmaceutical products currently in use are plant-derived, and approx. 75 % of these were discovered by examining the use of these plants in traditional medicine [6]. In the treatment of diabetes, more than 1000 traditional plants have been recorded, but only a small number of these have received scientific and medical evaluation to assess their efficacy [7,8]. The World Health Organization Expert Committee on Diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated [9,10]. Traditional anti-diabetic plants might, e.g., provide a useful source of new oral antihyperglycaemic compounds, in which the plant *Stevia rebaudiana* Bertoni (SrB) may be an option.

Extracts of the leaves of the plant *Stevia rebaudiana* Bertoni have been used for many years in traditional South American treatment of diabetes [11]. The plant was first discovered by the Paraguayan botanist, Moises Santiago Bertoni in 1899 who learned of its unique properties from the Paraguayan Guarani Indians [12]. The question arises, can compounds derived from *Stevia* be used in the treatment of type 2 diabetes? We have in our laboratory demonstrated that the diterpene glycoside, stevioside, isolated from SrB possesses anti-diabetic efficacy in animals

CHAPTER 4

Stevioside metabolism and transport in rats and humans

Eriko KOYAMA

Kashima Laboratory, Mitsubishi Chemical Safety Institute Ltd,
14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, Japan
Email: eriko36@lapis.plala.or.jp

Introduction

Stevioside, a sweetener extracted from the leaves of *Stevia rebaudiana* Bertoni, is the glycoside of the diterpene derivative including the aglycone steviol. The purpose of this study was to investigate intestinal degradation, absorption, and hepatic metabolism of stevioside using LC/MS/ESI or HPLC-UV analysis.

Results

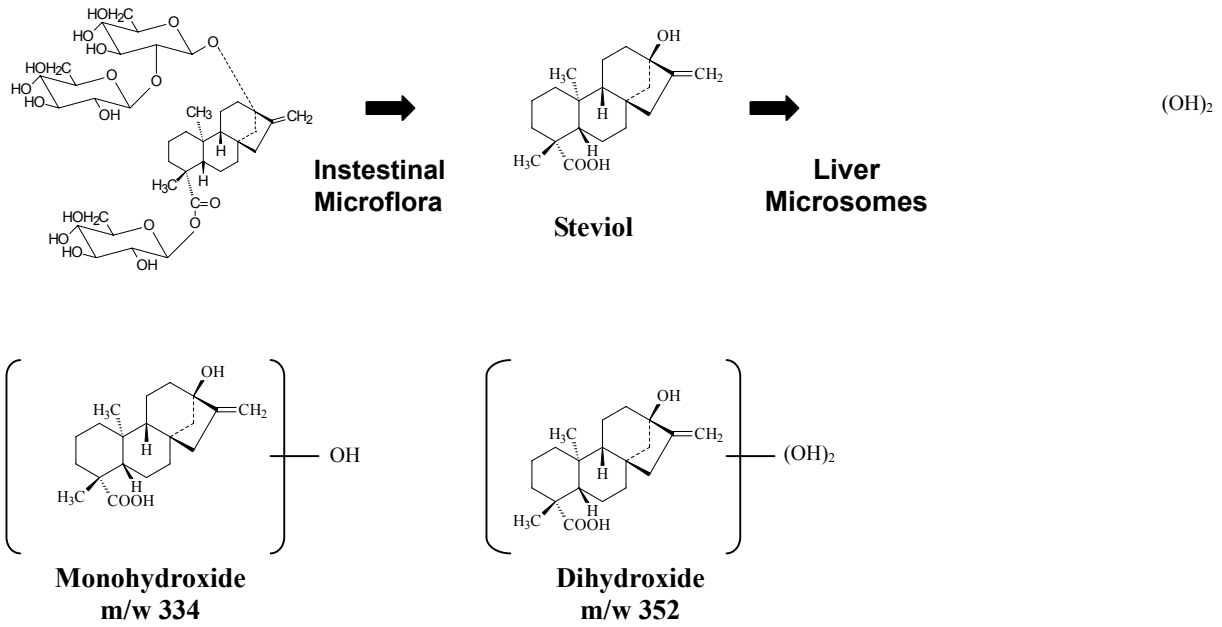
Degradation was examined by incubating stevioside or the aglycone steviol using pooled human fecal homogenates (obtained from five healthy volunteers) for 0, 8 and 24 hours under anaerobic conditions. Stevioside (0.2 mg/mL) were completely eliminated within 24 hours, whereas no degradation of steviol (0.08 [equimolar to stevioside] and 0.2 mg/mL) was found throughout the incubation period. Stevioside appeared to be hydrolyzed eventually to steviol by human intestinal microflora, consistent with the previous rat study[1].

Absorption was investigated by *in vivo* and *ex vivo* experiments using male SD rats. In an *ex vivo* experiment, stevia mixture components including stevioside, and steviol (5 mg/mL and 0.1 mg/mL, respectively) were incubated with everted sacs of rat intestine for 30 min under 95% O_2-5% CO_2. No absorption of stevia mixture

components including stevioside were observed (<0.2%), whereas the absorption of steviol (3%) was equivalent to approximately 70% of the salicylic acid positive control. As an *in vivo* experiment, the time-concentration profile of steviol was examined in male SD rats after a single oral dose of 45 mg/kg steviol or 125 mg/kg stevia mixture including stevioside (almost equimolar to steviol). Peak plasma concentration of steviol was observed 15 min after oral administration of steviol to rats, reflecting its rapid absorption. On the other hand, after oral administration of stevia mixture, steviol appeared in plasma at 2 hours and thereafter increased in a time-dependent manner over a further 8 hours. In combination with the degradation study, these *ex vivo* and *in vivo* findings suggest that stevia mixture/components are absorbed as steviol in the rat intestine after degradation by rat intestinal microflora has occurred.

Metabolism was examined by incubating steviol using rat or pooled human liver microsomes. This indicated that steviol was oxidized to monohydroxide (m/z 333) or dihydroxide (m/z 351) in both liver microsomes.

Stevioside



CHAPTER 5

The metabolism of stevioside by animals: chickens and pigs

Johan BUYSE[1] and Jan M.C. GEUNS[2]

[1]*Laboratory for physiology and Immunology of Domestic Animals*
Department of Animal Production
Katholieke Universiteit Leuven
Kasteelpark Arenberg 30
3001 Leuven, Belgium
Email: johan.buyse@agr.kuleuven.ac.be

[2]*Laboratory for Functional Biology*
Katholieke Universiteit Leuven
Kasteelpark Arenberg 31
3001 Leuven, Belgium
Email: jan.geuns@bio.kuleuven.ac.be

Introduction

Stevioside is the main sweet component in the leaves of *Stevia rebaudiana* (Bertoni) and tastes about 300 times sweeter than sucrose. *Stevia* leaves, stevioside and *Stevia* extracts are used as a natural sweetener in many countries of South America, North America, Japan and Korea. In these countries, powdered *Stevia* leaves or refined extracts are accepted as a dietary supplement. The advantages of stevioside are manifold, it is noncalorific, preserves dental health as sugar intake is reduced and is beneficial for diabetic and phenylketonurea patients and obese subjects.

Many papers have been published describing the safety of stevioside used as a sweetener (for reviews, see Geuns 2003 and this volume). However, in the

European Union, *Stevia* leaves and stevioside are still not approved for use in the food chain. There are some reports (e.g. Pezzuto *et al*., 1985) that steviol – the aglycon of stevioside – might have some mutagenic effects. Therefore, one of the most urgent problems to solve is the possible breakdown of stevioside into steviol or other metabolites *in vivo*. A second important issue is the question whether steviol, should it be produced, is taken up by the intestine and to what extent?

The present paper gives an overview of the *in vivo* and *in vitro* trials we conducted with high productive farm animal species: domestic chickens from different developmental stages (embryonic, growing, adult stage) and growing pigs in order to investigate the metabolism of stevioside and steviol.

Furthermore, anaerobic incubations were done with chickens and pig faeces to study the conversion of stevioside to steviol, and *in vitro* transport studies were done using human Caco-2 monolayers.

Trials with domestic chickens

1) Intubation experiments. In a first series of experiments broiler chickens (Cobb) were kept individually in digestibility cages and fed *ad libitum* a commercial grower diet (Geuns *et al*., 2003a). Under each cage, 70 cm below the bottom grid, a polystyrene container with solid carbon dioxide was placed. The excreta were collected quantitatively on a sheet of aluminium foil in direct contact with the solid CO_2. This method was used to avoid *ex vivo* bacterial decomposition of stevioside or steviol at room temperature. Three broiler chickens weighing about 0.9 kg were intubated into the crop with 643 mg stevioside (96 % purity) dissolved in 6 mL of 35% glycerol solution (714 mg/kg BW). To ensure that all stevioside was in solution, the stevioside/glycerol mixture was heated at 70°C and cooled to 40 °C before intubation. Immediately after intubation, the excreta were collected at regular time intervals, weighed, and stored frozen at –20°C after which they were freeze-dried before analysis of stevioside and steviol. A blood sample was taken from a wing vein using a heparinized syringe at 2, 4, 6, 8, 24 and 48 h after

CHAPTER 6

Bioavailability of stevioside from *Stevia rebaudiana* in human volunteers: preliminary report

P. SIMONETTI[1], C. GARDANA[1], L. BRAMATI[2] and P.G. PIETTA[2]

[1] *diSTAM - Division of Human Nutrition, University of Milan, Via Celoria, 2 - 20133 Milan, Italy;*
[2] *ITB-CNR, V.le f.lli Cervi, 96 – 20192 Milan, Italy*

Abstract

Stevioside is a natural compound extracted from *Stevia rebaudiana bertoni* leaves and used as natural sweeteners or dietary supplements for its sweetness intensity and not-caloric and not-cariogenic properties. The aim of this research was to investigate the stevioside bioavailability and its metabolic fate in human healthy volunteers, who received 375 mg stevioside oral single dose. At the beginning and at different times after stevioside administration, urine, faeces and plasma samples were collected, extracted and analysed for the presence of stevioside or its possible metabolites like steviol, steviol-16,17-α-epoxide and 15-α-hydroxysteviol by means of an LC-MS method developed for this study.

The results obtained are particularly interesting, since they prove that stevioside is absorbed and steviol-glucuronide is the only plasma metabolite found. Furthermore, steviol, steviol-16,17-α-epoxide and 15-α-hydroxy-steviol were not found in plasma, urine and faeces samples and steviol (stevioside aglycon) was found only in faeces samples.

Key words: Stevioside; bioavailability; humans; metabolism; mass spectrometry

Introduction

Stevia rebaudiana Bertoni (*Stevia*) is a perennial shrub that grows up to 1 m tall and its has leaves are 2-3 cm long. It belongs to the Asteraceae (Compositae) family, which is indigenous to the northern regions of South America (Brazil and Paraguay). The *Stevia* leaf contains from 6 to 18 % of stevioside, which is considered 300 times sweeter than sucrose at 0.4% sucrose concentration, 150 times sweeter at 4% sucrose, and 100 times sweeter at 10% sucrose concentration. Stevioside has been suggested to exert beneficial effects on humans health, including antihypertensive (Chan *et al.,* 2000, Lee *et al.,* 2001), antihyperglycaemic (Jeppesen *et al.,* 2002, 2000), antioxidant (Xi *et al.,* 1998), non cariogenic (Das *et al.,* 1992) and anti-human rotavirus (Takahashi *et al.,* 2001) activities. This sweetener is also thought to influence the glucose metabolism (Suanarunsawat and Chaiyabutr, 1997, Toskulkao *et al.,* 1995) and the renal function (Jutabha *et al.,* 2000). Moreover, several toxicological studies were carried out to verify the possible stevioside toxicological effects on mammalian species, and the results were recently reviewed (Smirnova, 2001, Geuns, 2000, Huxtable, 2002). The potential genotoxicity of *Stevia* extract and steviol was also evaluated using the comet assay (Sekihashi *et al.,* 2002). The results obtained showed that *Stevia* extract and steviol not have DNA-damaging activity in cultured cells and mouse organs. Indeed, regarding stevioside bioavailability and metabolism in humans the available data are very little and some aspects have not yet been fully elucidated. The metabolic fate of stevioside and its analogues has been studied extensively in animals, *in vitro* and *in vivo* models (Koyama *et al.,* 2003, Geuns *et al.,* 2003, Gardana *et al.,* 2003) but, to our knowledge, there is no paper describing the uptake of stevioside in humans and its metabolic fate.

CHAPTER 7

A short-term study of stevioside in healthy subjects

Elisabeth H.M. TEMME[1], Annelies VANKEIRSBILCK[1], Johan BUYSE[2]

and Jan M.C. GEUNS[3].

K.U. Leuven, [1]Faculty of Medicine, Department of Public Health, Division of Nutritional Epidemiology, Kapucijnenvoer 33-35, B-3000 Leuven, Belgium
[2]Faculty Agricultural and Applied Biological Sciences, Laboratory of Physiology and Immunology of Domestic Animals, Kasteelpark Arenberg 30, 3001 Leuven,
[3]Faculty of Sciences, Laboratory of Functional Biology,
Kasteelpark Arenberg 31, 3001 Leuven.

Address correspondence to:
Elisabeth HM Temme, PhD
K.U. Leuven, Department of Public Health
Division of Nutritional Epidemiology
Kapucijnenvoer 33-35
B-3000 Leuven, Belgium
Phone : +32 16 337015
Fax : +32 16 336884
E-mail: Liesbeth.Temme@med.kuleuven.ac.be

Summary

Stevioside is a natural plant glycoside isolated from the plant *Stevia rebaudiana*. It is 300 times sweeter than sugar but contains no calories. Stevioside would therefore be suitable for diets of e.g. diabetic and obese persons. In addition, studies suggested hypotensive and hypoglycaemic effects of stevioside when administered in a high dosage. This study was undertaken to evaluate the short-term effects on blood pressure, urinary excretion of electrolytes and blood glucose/insulin concentrations, in healthy subjects.

The study group consisted of 9 healthy subjects aged between 21 and 29 years. Over a period of 3 days, each subject was given capsules containing stevioside (250 mg) thrice daily. A blood sample was collected and blood pressure was measured before (after nocturnal fasting) and at different time-points after 3 days of stevioside. In addition, two 24-hour urine samples (before and after) were collected by the volunteer to evaluate the volume and concentrations of electrolytes.

The average systolic and diastolic blood pressure was 115 mmHg and 72 mmHg for the stevioside and 114 mmHg and 74 mmHg for the control condition, respectively. No significant differences were found between the stevioside and the control condition. Twenty-four hour urinary volume and urinary excretion of electrolytes was not significantly greater in the stevioside compared with the control condition. Mean blood glucose and insulin were 4.63 mmol/L and 5.9 mU/L for the stevioside and 4.60 mmol/L and 5.6 mU/L for the control condition, respectively, there being no difference between them.

Stevioside, when administered orally for three days in three 250 mg capsules, is not directly effective as a hypotensive or hypoglycaemic agent in healthy subjects, although it might stimulate water and sodium excretion *via* the urine. More information is needed on longer-term and post-prandial effects.

Key words Stevioside, steviol, blood pressure, glucose, insulin

Introduction

Stevioside is a natural sweet-tasting glycoside extracted from the plant *Stevia rebaudiana* Bertoni belonging to the *Compositae* family and native to Brazil and

CHAPTER 8

About the safety of stevioside used as a sweetener

Jan M.C. GEUNS[1], Johan BUYSE[2], Annelies VANKEIRSBILCK[3], Liesbeth TEMME[3]

[1]Laboratory of Functional Biology, KULeuven, Kasteelpark Arenberg 31, B-3001 Leuven; Tel.: +32-16-321510; Fax: +32-16-321509; e-mail:Jan.Geuns@bio.kuleuven.ac.be
[2]Faculty of Agricultural and Applied Biological Sciences, Laboratory of Physiology and Immunology of Domestic Animals, Kasteelpark Arenberg 30, B-3001 Leuven,
[3]Faculty of Medicine, Department of Public Health, Division of Nutritional Epidemiology, Kapucijnenvoer 33-35, B-3000 Leuven, Belgium.

Summary

Stevioside is a natural sweetener extracted from leaves of *Stevia rebaudiana* (Bertoni) Bertoni. Some of the more persistent rumours about possible harmful effects of stevioside eg. on male fertility and carcinogenicity, are discussed and subsequently refuted.

The metabolism of stevioside by volunteers has been studied. In the faeces only free steviol was found. Concentrations of free steviol or stevioside in blood or urine were below the detection limits. Steviol conjugates were found in blood and urine as typical excretion products.

A risk assessment was made taking the daily sugar consumption in Belgium as an example.

Introduction

The literature about *Stevia*, the occurrence of its sweeteners, their biosynthetic pathway and toxicological aspects were recently discussed (Geuns, 2002, 2003, 2004; Huxtable, 2002). Injection experiments or perfusion experiments of organs were considered as not relevant for the use of *Stevia* or stevioside as food additive, and, therefore, these studies were not considered. Although the literature proving the safety of stevioside is still increasing, many rumours about harmful effects are persistent. Therefore, a discussion is given of several "hot items" to refute the most persistent untruths and inventions about harmful effects. As additional metabolism studies of stevioside by humans were required, we performed such experiments with 10 healthy volunteers.

Stevioside and fertility

Planas and Kuć (1968) reported a decrease in the live birth rate of rats. However, their results were refuted by Shiotsu (1996) who did more reliable experiments with many more animals using methods as similar as possible to the methods used by Planas and Kuć (1968). No effects on general condition, body weight, water consumption, and live birth rate or litter size were found after the administration of *Stevia* extracts. Planas and Kuć (1968) also clearly stated that only the female animals received *Stevia* extract and that the males never received it. Therefore, the claims that male fertility was influenced, is no more than fantasy. Many other papers could not demonstrate a harmful effect on male or female fertility (for a review see Geuns, 2004).

Melis (1999) suggested a possible decrease of fertility in young male rats fed for 60 days with very concentrated extracts of *Stevia* leaves. Extracts of 2.668 g dry leaves (~ 26.68 g fresh wt.) were fed daily to rats weighing 50 g: i.e. on a fresh

CHAPTER 9

REVIEW: The safety of stevioside used as a sweetener

Jan M.C. GEUNS

Laboratory of Functional Biology, KULeuven,
Kard. Mercierlaan 92, B 3001 Leuven
Tel.: +32-16-321510; Fax: +32-16-321509
e-mail: Jan.Geuns@bio.kuleuven.ac.be

ABSTRACT: Stevioside is a natural sweetener extracted from leaves of *Stevia rebaudiana* (Bertoni) Bertoni. The literature about *Stevia*, the occurrence of its sweeteners, their biosynthetic pathway and toxicological aspects are discussed. Injection or perfusion experiments of organs are considered as not relevant for the use of *Stevia* or stevioside as food additive, and therefore these studies are not included in this review.

The metabolism of stevioside is discussed in relation to the possible formation of steviol in both animals and man. Different mutagenicity studies as well as studies on carcinogenicity are discussed. Acute and sub-acute toxicity studies revealed a very low toxicity of *Stevia* and stevioside. Fertility and teratogenicity studies are discussed as well as the effects on the bio-availability of other nutrients in the diet.

The conclusion is that stevioside is safe when used as sweetener. It is suitable for both diabetics, and PKU patients, as well as for obese persons intending to lose weight by avoiding sugar supplements in the diet. No allergic reactions seem to exist.

Abbreviations

ADI: Allowable daily intake; BW: Body weight; CHL: Chinese hamster lung fibroblast cell line; Glc: Glucose; ICH: International Council of Harmonisation; JECFA: Joint FAO/WHO Expert Committee on Food Additives; LD_{50}: Lethal dose at which 50% of the animals die; NOEL: No-observable effect level; OECD: Organisation for economic co-operation and development; PKU: Phenylketonuria; Rha: Rhamnose; Xyl: Xylulose.

Introduction

Stevia rebaudiana (Bertoni) Bertoni is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil). It is known to the Guarany people, native to these regions since time immemorial, by several names all of which refer to the sweet taste of the leaf, and especially to its use in "mate" tea (*Ilex paraguariensis*). It is often referred to as "the sweet herb of Paraguay".

Stevioside, the main sweet component in the leaves of *Stevia rebaudiana* (Bertoni) Bertoni tastes about 300 times sweeter than sucrose (0.4% solution). Structures of the sweet components of *Stevia* occurring mainly in the leaves are given (Fig. 1). Their content varies between 4 and 20 % of the dry weight of the leaves depending on the cultivar and growing conditions. In most crops grown in the field the sweetener content is about 10% of the leaf dry weight. Stevioside **3** is the main sweet component. Other compounds present, but in lower concentration, are: steviolbioside **2**, rubsoside **4**, rebaudioside A **5**, B **6**, C **7**, D **8,** E **9**, F **10** and dulcoside A **11** (Kennelly, 2002, Starrat *et al*., 2002). The presence of steviolbioside and rebaudioside B in extracts might be due to artifacts of the extraction procedure (refs. in Kennelly, 2002).