Forensic Science International 240 (2014) e1–e6

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Case Report

Purple discoloration of the colon found during autopsy: Identification of betanin, its aglycone and metabolites by liquid chromatography–high-resolution mass spectrometry

Andreas T. Roemmelt [a], Sabine Franckenberg [b], Andrea E. Steuer [a], Thomas Kraemer [a,*]

[a] Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland
[b] Department of Forensic Medicine and Imaging, Zurich Institute of Forensic Medicine, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 13 November 2013
Received in revised form 10 March 2014
Accepted 15 March 2014
Available online 26 March 2014

Keywords:
Purple colon
Betanin
Betanidine
Metabolites
LC–HRMS

ABSTRACT

During autopsy of a 38-year-old man the forensic pathologist noted an atypical purple discoloration of the colon membrane. Hypothesis was that the discoloration could have been caused by ingestion of red beetroot. In order to exclude other toxicological causes for this finding and to analytically verify this hypothesis, colon membrane, blood and urine were screened not only for the typical forensically relevant substances but also for the main chromophoric beetroot compounds employing liquid chromatography–high-resolution mass spectrometry (LC–HRMS). Betanin (*m*/*z* 551.1495) and its aglycone betanidin (*m*/*z* 389.0973) were found in the extracts of colon membrane and urine. Betanin was detected in whole blood, and urinary analysis additionally revealed two metabolites: betanidin glucuronide (*m*/*z* 565.1294) and betanidin sulfate (*m*/*z* 469.0541) – showing the same fragmentation pattern as betanidin after the characteristic neutral loss of *m*/*z* 176.0315 and *m*/*z* 79.9554 for glucuronic acid and sulfate, respectively. This is the first time that betacyanins could be analytically confirmed as cause for a purple discoloration of the colon. Urine analysis further revealed that besides betanin itself betanidin phase II metabolites could be detected in human urine.

1. Introduction

Red beetroot (*Beta vulgaris*, *Amaranthaceae*) is a widely consumed vegetable in Europe and per capita consumption is about 8.2 kg per year. The consumption of this vegetable is en vogue because of its alleged positive effects on human health. Beetroot or red beet juice (RBJ) ingestion has shown inverse effects on cardiovascular diseases, cancer and other chronic diseases [1]. Especially the modulation of neutrophil oxidative metabolism and its consequences, such as DNA damage and apoptosis can be responsible for betanin's anti-inflammatory and anticarcinogenic activity [2,3]. In some areas RBJ or the vegetable is used as an adjunctive therapy in cancer treatment. Since red beetroot and its juice are also rich in nitrate it is very popular as dietary nitrate supplementation in competitive sport to improve performance [4–7]. Red beetroot contains unique bioactive compounds, called betalains. They can only be found in red beetroot and cactus pear [3]. Betalains are a very heterogeneous group which consists of red violet betacyanins and yellow betaxanthins. Both groups are quaternary ammonium derivatives of 4-vinyl-5,6-dihydropyridine-2,6-dicarboxylic acid (Fig. 1) and are readily soluble in water. Further ingredients are for example flavonoids and other phenolic structures. Among the betalains in red beetroot betanin and its C15 isomer isobetanin are the most prevalent compounds. The concentration of betanin in red beetroot can reach 300–600 mg/kg [8]. Betanin has a positive nitrogen in a polyene system which gives the fruit its characteristic red violet color. Jackman et al. [9] showed that the color properties of betanin are stable over a broad pH range from 3 to 7. Betanin is also known for a long time as a safe food colorant (E162) for groceries [10] or to color tablets in pharmaceutical companies and in illegal drug production. It was also observed that after red beetroot or juice consumption the color of urine can turn into slightly reddish, also known as beeturia [11]. However, only 10–14% of humans excrete reddish urine after ingestion [12]. This discoloration is caused by betalains and their metabolites. Discoloration of the colon seen during autopsy has been reported in the literature and consumption of red beetroot

* Corresponding author at: Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University of Zurich, Winterthurerstrasse 190/52, CH-8057 Zurich, Switzerland. Tel.: +41 446355641; fax: +41 446356852.
E-mail address: thomas.kraemer@irm.uzh.ch (T. Kraemer).

http://dx.doi.org/10.1016/j.forsciint.2014.03.016
0379-0738/

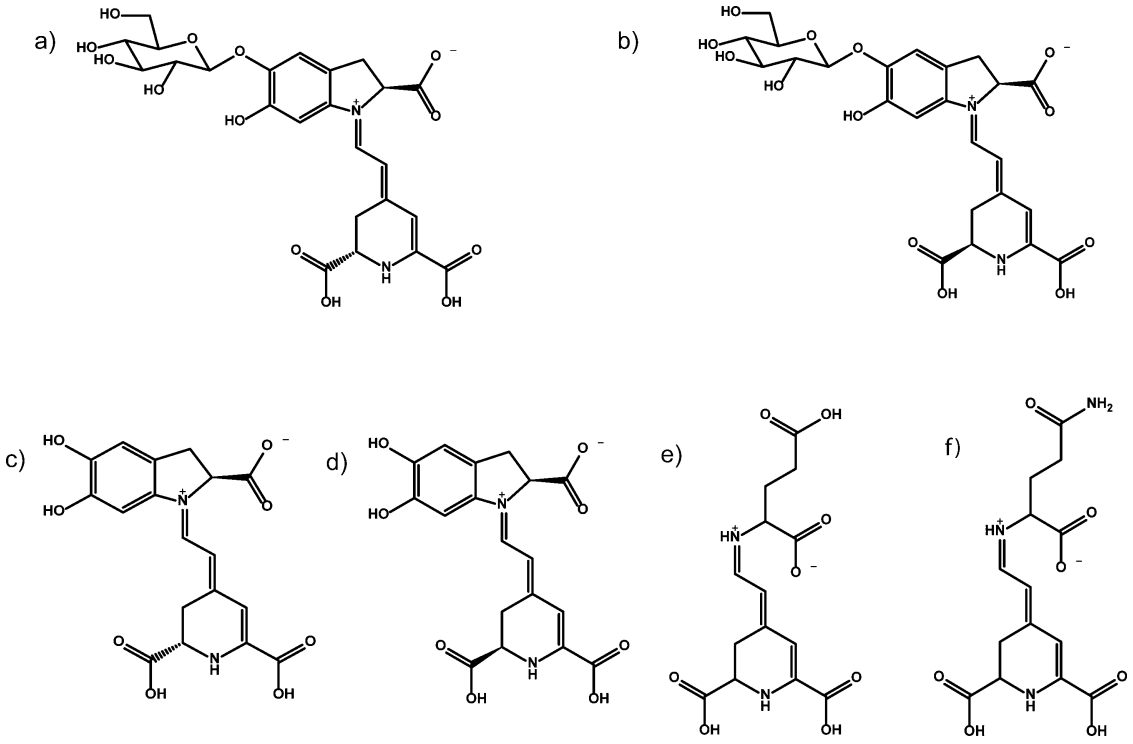

Fig. 1. Chemical structures of important betalains: betanin (a), isobetanin (b), betanidin (c), isobetanidin (d), vulgaxanthin I (e) and vulgaxanthin II (f).

has been suspected to be the reason for that. However, an analytical proof has been missing so far [13].

2. Case

A 38-year-old male was found with low vital functions in his living room by his girlfriend. Under resuscitation he was immediately transported to the hospital but died on the way. Hours before the incident he had visited a techno festival in the city of Zurich. Since there was no obvious reason of death, the district attorney initiated an autopsy and a forensic toxicological analysis. The toxicological analysis revealed several licit and illicit drugs (Table 1). Multiple drug intoxication was claimed to be the presumptive cause of death. However, these results could not explain the observation of a purple discoloration of the colon transversum and descendens, found during autopsy (Fig. 2). Although there are hundreds of autopsies in Zurich each year, such a discoloration had never been observed before. Hypothesis was that red beetroot might have caused the discoloration. To ensure that the purple discoloration was actually caused by non-toxic red beetroot, presence of red beetroot ingredients should be confirmed by liquid chromatography–high-resolution mass spectrometry (LC–HRMS).

Table 1
Results of the toxicological analysis of the blood.

Drugs	Blood concentration
THC	1.6 μg/L
THC-OH	0.9 μg/L
THC-COOH	28 μg/L
Methadone	180 μg/L
Cocaine	13 μg/L
Norcocaine	43 μg/L
Methylecgonine	43 μg/L
Benzoylecgonine	220 μg/L
Amphetamine	260 μg/L
MDMA	48 μg/L
Alprazolam	150 μg/L
Citalopram	1000 μg/L
GHB	7.5 mg/L

3. Materials and methods

3.1. Chemicals and reagents

Betanin as reference standard, water (HPLC grade), acetonitrile, formic acid, ammonium formate, diethylether, potassium hydroxide, carbitol, *N*-methyl-*N*-(*p*-tolylsulfonyl)nitrosamide and a diazomethane generator were obtained from Sigma–Aldrich (Buchs, Switzerland). Dialysis membrane Spectra Por came from Spectra Laboratories Inc., California. The 0.22-μm syringe filters were obtained from TPP Techno Plastic Products AG (Trasadingen, Switzerland). Diazomethane was synthesized according to the procedure of Fales et al. [14].

3.2. Sample collection and preparation

14 g of colored colon was excised and homogenized in a mixer. The whole sample was dialyzed in methanol–water (50:50) solution over 24 h employing a Spectra Por dialysis membrane. The dialyzed solution was filtered through a 0.22 μm pore size filter. This solution was diluted tenfold and measured by vis spectroscopy in a 1 cm cuvette in the wavelength range between 500 nm and 800 nm. For LC–HRMS analysis 10 μL were injected as described below.

For the detection of metabolites of betanin in urine, 0.5 mL of urine was diluted with 1 mL of ice cold acetonitrile. The solution was centrifuged for 5 min at 12.500 rpm. The supernatant was filtered through a 0.22 μm pore size filter to remove remaining suspended particles and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in 50 μL mobile phase 5 mM ammonium formate and acetonitrile with 0.1% formic acid (50:50, v/v). Then 10 μL were injected into the LC–HRMS system.