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Heat-induced formation of mepiquat by decarboxylation of pipecolic acid and its betaine derivative. Part 1: Model system studies

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ABSTRACT

This study describes, for the first time, the role of pipecolic acid betaine and pipecolic acid, naturally present in some foods, in the formation of the plant growth regulator N,N-dimethylpiperidinium (mepiquat) under dry thermal conditions. The formation of mepiquat and intermediate compounds was investigated in model systems using high performance liquid chromatography-quadrupole/time-of-flight mass spectrometry. Mepiquat is released with a yield of up to 0.66 mol% after thermal treatment (>150 °C) of pipecolic acid betaine. Similar conversion rates are attained with the congener piperidine-2-carboxylic acid (DL-pipecolic acid), albeit in the presence of alkylating agents, such as choline, glycine betaine or trigonelline, that are fairly widespread in food crops. These new pathways to mepiquat indicate that the occurrence of low levels of this thermally induced compound is probably more widespread in processed foods than initially suspected (see Part 2 of this study on the occurrence of mepiquat in selected foodstuffs).

1. Introduction

N,N-dimethylpiperidinium (mepiquat, Fig. 1) is a well-known plant protection product commonly used as a plant growth regulator in agriculture, usually as the chloride salt, which acts by inhibiting the synthesis of gibberellins (Rademacher, 2000). Recent studies showed that mepiquat is formed at low mg/kg amounts during the coffee roasting process and can consequently be detected in roast and ground coffee, as well as soluble coffee, reaching levels up to 1.4 mg/kg (Wermann et al., 2014). Mepiquat (or its chloride salt) is not registered for use on coffee, and should therefore not be detectable in green coffee beans. Mepiquat was also detected in roasted barley at amounts up to 0.64 mg/kg, corroborating that mepiquat is a process-induced compound, formed naturally during cooking practices, such as roasting and toasting (Wermann et al., 2014).

In essence, mepiquat arises from a Maillard-type reaction that requires free lysine, a reducing sugar and an alkylating agent, heated under dry conditions (Bessaire, Tarres, Delatour, & Stadler, 2014; Hammel, Dubois, Delatour, & Stadler, 2014). The cyclization of lysine in the presence of a reducing sugar furnishes piperidine (Nikolov & Yaylayan, 2010), followed by a nucleophilic displacement reaction in which the N-substituent is transferred from a quaternary nitrogen to the nucleophile (piperidine), at temperatures typically around 230 °C. So far, trigonelline, choline, and glycine betaine have been identified as possible methylating agents (Bessaire et al., 2014; Hammel et al., 2014; Stadler, Varga, Hau, Vera, & Welti, 2002).

Trigonelline (N-methylnicotinic acid) is a quaternary alkaloid found in a wide range of plants, including the plant families *Poaceae* (1.08–31.1 μg/g fresh weight), *Asteraceae* (12.4–41.4 μg/g fresh weight) and *Fabaceae* (166–8004 μg/g fresh weight) (Corol et al., 2012; Matsui, Yin, Yamanaka, Iwasaki, & Ashihara, 2007). More importantly, trigonelline is known to be the second most abundant alkaloid in coffee (*Rubiaceae*) after caffeine (Stadler, Varga, Milo, et al., 2002). Based on the data available in the scientific literature, contents of trigonelline in coffee beans range from 7.7 to 14.8 mg/g dry weight for *Coffea arabica*, and 5.2 to

Abbreviations: PipBet, pipecolic acid betaine; PipAc, , pipecolic acid.

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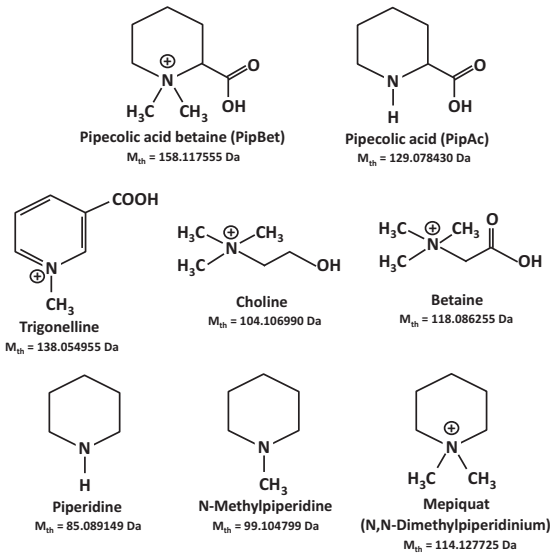

Fig. 1. Chemical structures and theoretical exact masses (M_{th}) of pipecolic acid betaine and pipecolic acid (upper line), methylating agents (middle line) and piperidine derivatives (bottom line).

15.5 mg/g dry weight for *C. canephora* (Campa et al., 2004; Koshiro, Zheng, Wang, Nagai, & Ashihara, 2006; Lang, Yagar, Eggers, & Hofmann, 2008; Matsui et al., 2007; Perrone, Donangelo, & Farah, 2008; Stennert & Maier, 1994). Both choline and glycine betaine are known to occur in various cereals in the range 0.2–1.1 mg/g and 0.4–12.9 mg/g for choline and glycine betaine, respectively (Corol et al., 2012; Likes, Madl, Zeisel, & Craig, 2007).

Not many reports are available on the occurrence of pipecolic acid (PipAc) and pipecolic acid betaine (PipBet) in foods. Fujita and coworkers identified PipAc in various foodstuffs, including fermented beverages, processed vegetables, dairy products and cheese (Fujita, Fujita, Kodama, Hada, & Higashino, 2003). PipAc is generated by the metabolism of lysine induced by mammalian and bacterial enzymes (Chang, 1976, 1978). The betaine congener PipBet is synthesized from PipAc in plant cells, which is a common biochemical response to stress phenomena (Servillo et al., 2012). In fact, the presence of both PipAc and PipBet have been reported in plant species belonging to the genus *Citrus* (Servillo et al., 2012).

The objective of this study was to investigate the ability of both PipAc and PipBet to generate mepiquat under dry thermal conditions. Due to the structural similarity of PipAc and PipBet with mepiquat, we postulated that a thermally-driven decarboxylation of these two precursors would afford mepiquat either directly (from PipBet), or indirectly via piperidine. Model system studies included PipBet or PipAc, heated alone or in the presence of an alkylating agent (trigonelline, choline or glycine betaine). Analysis of the reaction mixtures by liquid chromatography-high resolution mass spectrometry enabled the identification of the compounds involved in the chemical pathways.

2. Materials and methods

2.1. Chemicals and reagents

Pipecolic acid (PipAc, >98%), betaine hydrochloride (glycine betaine, >99%), choline chloride (>99%), trigonelline hydrochloride (>98%), piperidine (>99%), N,N-dimethylpiperidinium chloride (mepiquat chloride) and mepiquat iodide-(methyl-d_3) (d_3-mepiquat, isotopic purity >98%) were purchased from Sigma Aldrich (Buchs, Switzerland). Pipecolic acid betaine (PipBet) was obtained by customized synthesis from Atlanchim Pharma (Saint Herblain, France) at a purity >95%. The structure and purity of PipBet were confirmed by MS and NMR analyses performed by Atlanchim Pharma (Saint Herblain, France). Acetonitrile (Optima™ LC/MS) and water (Optima™ LC/MS) were supplied by Fisher Scientific (Reinach, Switzerland). Methanol (gradient grade for LC) and ammonium acetate were obtained from Merck (Darmstadt, Germany).

2.2. Model systems

Water (20 µl) was added to PipBet (10 mg) in a pyrolysis tube (ASS Glass, USA) that was subsequently tightly closed. In the case of PipAc, either 57 mg of trigonelline hydrochloride, 58 mg of choline chloride or 64 mg of betaine hydrochloride were added to 54 mg of PipAc and mixed into water (50 µl) prior to heating in a tightly closed pyrolysis tube. The tubes were heated from 150 °C to 250 °C in a temperature-controlled oil bath for 30 min. For the kinetic study, the tubes with PipBet or PipAc in the presence of alkylating agents were heated at the optimal temperature in a temperature-controlled oil bath from 0 to 180 min.

At the end of the reaction time, the pyrolysis tubes were cooled down to room temperature and 10 ml of a mixture methanol/water (50/50, v/v) was added. After shaking on a vortex and sonication for 10 min, a 50 µl aliquot of the resulting mixture was sampled and diluted into 950 µl of methanol. For quantification purposes, 50 µl of the aliquot were diluted into 940 µl of methanol and 10 µl of mepiquat-d_3 (4.8 µg/ml) added. The resulting mixture was filtered (Nylon, 0.22 µm, VWR) prior to analysis by LC-Q/ToF.

2.3. Analysis by LC-Q/ToF

HPLC analysis was performed on a hydrophilic–lipophilic (HILIC) column Xbridge™ BEH HILIC 2.5 µm, 2.1 × 100 mm (Waters, Dublin, Ireland) using an Agilent 1200 series binary pump device and an autosampler HiP-AKS SL + System from Agilent Technologies (USA). The mobile phase comprised of acetonitrile/ammonium acetate 10 mM, 95/5 (solvent A) and acetonitrile/ammonium acetate 10 mM, 50/50 (solvent B). The gradient applied was: from 0 to 6.0 min, a linear gradient from 100% solvent A to a mixture 50/50 (ratio A/B); from 6.0 to 8.0 min, a linear gradient from a solvent A/B mixture at 50/50 to 100% solvent B; from 8.0 to 8.5 min, mobile phase at 100% solvent B. The column was then equilibrated at 100% solvent A from 8.5 to 14.5 min. The column temperature was set at 40 °C with a solvent flow rate of 300 µl/min. A volume of 5 µl of sample was injected onto the column for LC-Q/ToF analysis.

Detection was carried out on an UHD Accurate-Mass Q/ToF from Agilent Technologies (USA) equipped with an AJS ESI source operating in the positive ionization mode. Acquisitions were recorded either in full scan (Full MS) or product ion (Targeted MS/MS) modes. The ionization parameters were: voltage: 3.1 kV; gas temperature: 300 °C; sheath gas temperature: 350 °C; nebulizer: 35 psi; fragmentor: 163.5 V for targeted MS/MS mode and 108 V for full-scan mode; skimmer: 48 V; the collision energy for mepiquat and mepiquat-d_3: 20 V. The acquisition time for targeted MS/MS mode was 3.5–9.0 min with a mass range defined at m/z 50–300. The platform was calibrated was a mass accuracy <2 ppm.

The exact masses of the compounds were calculated based on the elemental and electron masses hereafter: ^{12}C: 12.000000; ^{1}H: 1.007825; ^{16}O: 15.994915; ^{14}N: 14.003074; and electron: 0.000549 (Ferrer & Thurman, 2007; Peiser et al., 1984). Single-exact mass chromatographic profiles were extracted from the full-scan recorded data with a mass window defined at 10 ppm. Theoretical masses are reported with six digits after the decimal place, while four digits were used for the measured masses.