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W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
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U. FLENKER, W. SCHÄNZER:
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U. Flenker and W. Schänzer

$^{13}C/^{12}C$-Ratios in Caffeine from Different Sources and in Urinary Excreted Caffeine

Institute of Biochemistry, German Sports University Cologne

Abstract

We provide a method to analyze $^{13}C/^{12}C$-ratios of caffeine of different origin and of caffeine excreted in urine. Results indicate that clear isotopic signatures exist with respect to synthetic or plant origin. Caffeine excreted in urine still seems to bear this signature.

1 Introduction

Caffeine does not belong to the substances prohibited in sports principally. However there is a threshold level of 12μg caffeine per ml urine to cope with usual consumption habits on the one hand and to avoid excessive abuse by athletes on the other. Apart from the actual concentration of caffeine in urine, it is evidently of interest to obtain information about the source of this alkaloid. An increasing number of pharmaceutical and nutritional products with caffeine as additive is available. Measurement of isotope ratios usually is the method of choice to handle analytical questions like these. The present study is based on this approach and employs stable carbon isotopes.

2 Methods

2.1 Urine Samples

18 urines (U 1–18) taken from routine samples showing significant concentrations of caffeine were analyzed. One urine contained 25μg caffeine per ml and caused follow up studies. The other urines contained less than 12μg caffeine per ml. 3ml of each urine were analyzed.

Sample preparation was performed in the following manner: 1) Add 3ml of tertiary butylmethyl ether (TBME), 2) add $\approx$ 3g Na_2SO_4, 3) shake for $\approx$ 15min, 4) centrifugation and removal of organic layer, 5) evaporate organic layer to dryness, 6) take up residue in 100μl methanol.

Measurements were performed by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). 1μl of the obtained solution was injected into the device. Hardware and hardware setup was as described in [1]. The chromatographic column was a

M&N Optima-δ3 (Macherey & Nagel, Düren, Germany), 0.25mm inner diameter, 0.25μm film. Temperature was kept isothermal at 60 °C for 90s, 30K/min to 190 °C, 10K/min to 250 °C, 30K/min to 310 °C and again kept isothermal for 2min. Single measurements were performed.

2.2 Beverages and Pharmaceutical Products

Four "energy drinks" (ED 1–4), one "soft drink" (SD) and four pharmaceutical products (PP 1–4) containing caffeine were analyzed. These sources were expected to contain synthetic caffeine. One coffee product (CF), one green tea (GT) and one black tea product (BT) were investigated as representatives of caffeine from plant sources. 1ml of beverages were analyzed according to the method described above, with the exception that 1μl of the TBME layer directly was injected into the GC. Pharmaceutical products were ground, dissolved in water, treated according to the method for urine and finally were injected into the GC after appropriate dilution. Five replicate measurements were performed on all beverages and pharmaceutical products. Means and 99% confidence intervals were calculated for the resulting δ^{13}C-values.

3 Results

Figure 1 shows a chromatogram obtained from a routine urine sample. Typically chromatograms showed far less background and hardly any coelution. Extracts from beverages and pharmaceutical products showed even better chromatography.

Figure 2 summarizes the measured $^{13}C/^{12}C$-ratios of caffeine from all samples. A comparison to data from literature [2] is included as well.

4 Discussion

Considering the data of Weilacher and coworkers [2] and those of the present study a clear source assignment can be performed for caffeine based on $^{13}C/^{12}C$-measurements. Obviously caffeine found in urine still bears this isotopic signature. Carbon isotope ratios of caffeine from urine samples showing concentrations below the threshold level of 12μg/ml are in accordance with our data for caffeine from tea and coffee. They are also in accordance with the corresponding data from [2]. The positive urine sample shows an isotopic signature typical for synthetic caffeine, again based on our data and those from [2]. Follow up studies revealed that the athlete consumed high ammounts of an "Energy Drink". The product was identical to ED 1 in our study.

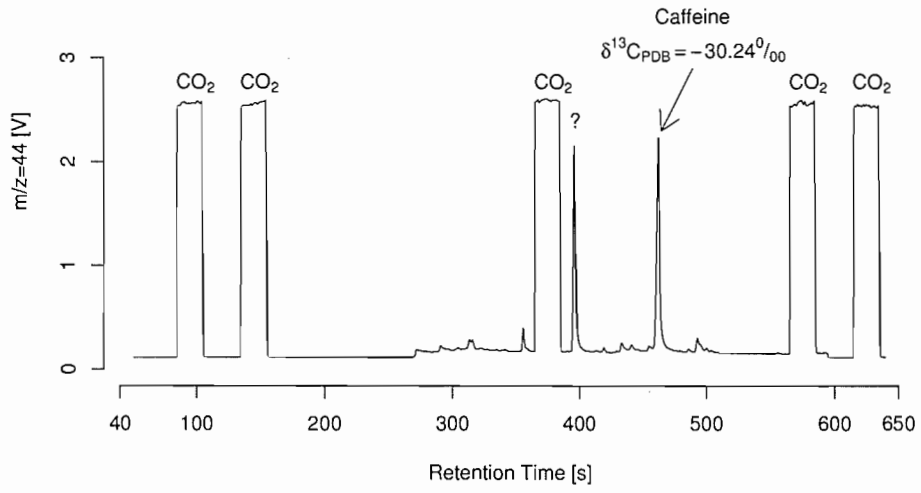

Figure 1: Chromatogram obtained from urine sample #206/2001 by GC/C/IRMS. The caffeine peak is annoted and shows an δ^{13}C-value of -30.24 $^0/_{00}$ *vs.* PDB. The question mark indicates an unknown compound that could be observed from time to time. "CO_2" indicates a reference gas pulse.

References

[1] U. Flenker, S. Horning, E. Nolteernsting, H. Geyer, and W. Schänzer. Measurement of $^{13}C/^{12}C$-ratios to confirm misuse of endogenous steroids. In W. Schänzer, H. Geyer, A. Gotzmann, and U. Mareck-Engelke, editors, *Proceedings of the Manfred Donike Workshop*, number 6 in Recent advances in doping analysis, pages 243–256. Sport und Buch Strauß, Köln, 1999.

[2] T. Weilacher, G. Gleixner, and H.-L. Schmidt. Carbon isotope pattern in purine alkaloids: A key to isotope discriminations in C_1 compounds. *Phytochemistry*, 41(4):1073–1077, 1996.

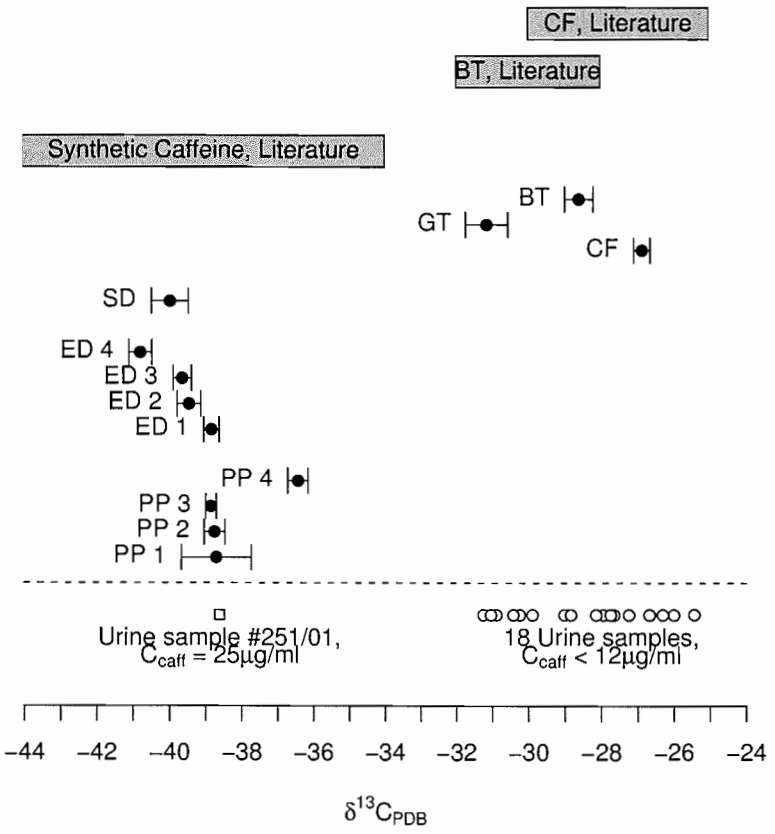

Figure 2: δ^{13}C-values of caffeine from 18 urine samples, four "energy drinks" (ED 1–4), one "soft drink" (SD) and four pharmaceutical products (PP 1–4). Error bars indicate 99% confidence intervals calculated from 5 repeated measurements. Rectangles indicate data from literature [2]. BT: black tea; CF: coffee