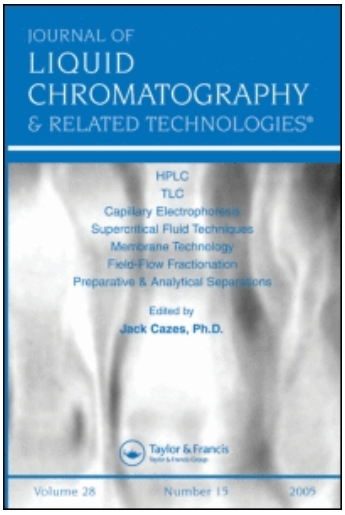


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# The Identification of 7-Methylxanthine in Cacao Products

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# The Identification of 7-Methylxanthine in Cacao Products

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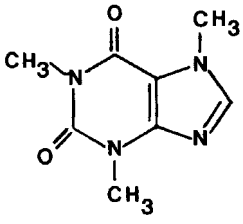
## ABSTRACT

High performance liquid chromatography has been employed to separate and identify 7-methylxanthine in aqueous extracts of defatted chocolate liquor. The alkaloid was identified by comparison of its behavior in a variety of solvent systems with that of an authentic sample, and through absorbance ratios at 254 and 280 nm.

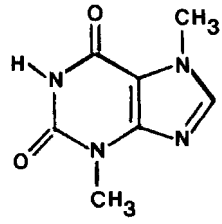
## INTRODUCTION

The presence of xanthine alkaloids in chocolate and cocoa, especially caffeine, theobromine, and theophylline (Figure 1), has been firmly established (1,2,3).

Recently HPLC has been used to quantify these major xanthines in chocolate and cocoa (4,5). This has fostered further attempts to detect the presence of other xanthine bases in these materials. In this study HPLC has



CAFFEINE



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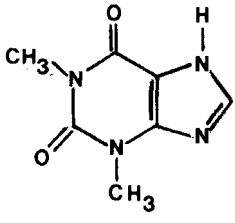

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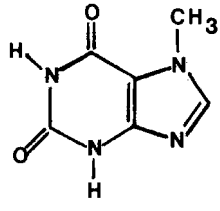

7-METHYLXANTHINE

Figure 1



been employed to separate and identify a minor xanthine alkaloid, 7-methylxanthine, in aqueous extracts of defatted chocolate liquor.

Carbon 14 tracer studies by Ogutuga and Northcote in 1970 suggested that 7-methylxanthine is an integral part of the biosynthetic pathway leading to the production of theobromine and caffeine in tea. By analogy, we may assume that 7-methylxanthine is a potential intermediate in the pathway leading to theobromine in cocoa.

## Sample Preparation

Samples of chocolate liquor were obtained from the Hershey Chocolate Company. A liquor sample (10 g) was defatted with two 75 ml portions of petroleum ether, and allowed to dry. When no residual solvent was evident,

2.50 g of the sample was dispersed in distilled water (50 g) and the mixture was boiled gently, with stirring, for 30 minutes. The sample was allowed to cool to room temperature, and brought to the original weight by addition of water. After filtration through Whatman No. 41 paper, or the equivalent, aliquot was filtered through a Swinney syringe using a 0.45μ filter, and the resulting filtrate was used for analysis.

Chromatography

The chromatographic system consisted of an M6000A Solvent Delivery System, U6K Injector, two M440 Ultraviolet Detectors (254 nm and 280 nm), all from Waters Associates, and two 10 mv recorders. The following 300x4.0 mm (I.D.) columns were used: μBondapak $C_{18}$ (10 μm particle size, Waters Associates) and Spherisorb ODS (10 μm particle size, HPLC Technology, Inc.). The mobile phases used consisted of 0.01M $H_3PO_4$ or 0.001M $H_3PO_4$ and HPLC grade THF, adjusted to various pH values by careful addition of NaOH solution (50%).

Standards

Standard 7-methylxanthine (Sigma Chemical Co.) was dissolved in distilled water to make a concentration of 0.1 μg/μl. The solution was filtered through a Swinney syringe system using a 0.45μ filter prior to use.



Table 1

Retention Times for Standard and Sample

| | | | Retention Time, minutes | |
|---|---|---|---|---|
| Column | Mobile Phase | pH | Standard | Sample |
| μBondapak $C_{18}$ | 0.5% THF in 0.01M aqueous phosphate | 3.0 | 32.1 | 32.2 |
| μBondapak $C_{18}$ | 0.5% THF in 0.01M aqueous phosphate | 6.0 | 36.0 | 35.8 |
| μBondapak $C_{18}$ | 0.5% THF in 0.001M aqueous phosphate | 4.0 | 33.0 | 33.0 |
| Spherisorb | 0.5% THF in 0.01M aqueous phosphate | 6.0 | 30.4 | 30.5 |

Table 2

Absorbance Ratios for Standard and Extract at pH 6.0

| Sample | Absorbance Ratio 254/280 nm |
|---|---|
| 7-Methylxanthine | 0.92 |
| Defatted liquor extract | 0.96 |

Analyses

Standard and extract solutions were injected successively into the HPLC using mobile phases at various pH values. Absorbance ratios at 254/280 were determined at pH 6.0 (± 0.01). The flow rate throughout the analyses was 0.5 ml per minute.

Results

The results indicate that the peak of interest is due to 7-methylxanthine.(7) The data in Table 1 show the retention time for base line resolutions of standard and sample at various pH values.

Absorbance ratioing (7) for the standard and samples indicates the peak of interest to be 7-methylxanthine.



## CONCLUSION

The presence of 7-methylxanthine in defatted chocolate liquor extract has been established by HPLC. These data support the contention of Ogutuga and Northcote (6) that 7-methylxanthine is a metabolic intermediate in the pathway leading to caffeine.

Further studies are underway aimed at the identification and quantitation of other minor xanthines in similar liquor extracts.

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