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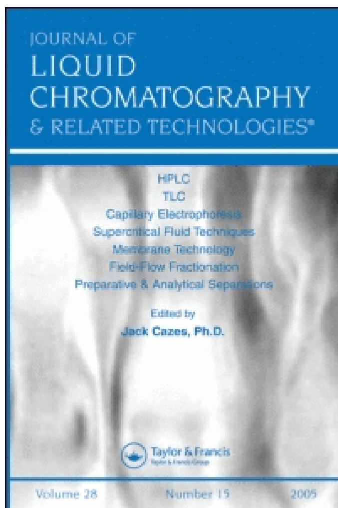

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THE IDENTIFICATION OF URIDINE IN CACAO EXTRACTS

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ABSTRACT

Uridine has been identified as a minor component of defatted Ecuadorian cacao liquor through reverse phase HPLC of aqueous extracts. The nucleoside was identified by comparison of its behavior in a variety of mobile phases and column types as well as its absorbance ratios at 245 and 270 nm. This work has extended the previously reported methodology to include pyrimidine nucleosides.

INTRODUCTION

We have previously reported the presence of 7-methylxanthine, adenine, and adenosine in aqueous defatted cacao liquor extracts, as identified through

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HPLC methodology.[1,2,5] The implication of such compounds in the biogenesis of xanthine bases in cacao is an extension of earlier investigations of Ogutuga and Northcote into similar biosyntheses in tea plants.[3] We have attempted to define a methodology with which to identify similar biosynthetic pathways in *Theobromo cacao* using techniques recently available and potentially more sensitive. With the identification of uridine in cacao extracts the methodology has been expanded to the pyrimidine bases using the same sampling techniques that were applied to purine bases.

Sample Preparation

Finely divided defatted Ecuadorian cacao liquor (2.5 g), obtained by two successive extractions with petroleum ether, was extracted with HPLC grade water (50 g) by heating to a low boil for 30 minutes with moderate stirring. After cooling the sample was brought to its original weight by the addition of HPLC grade water, and gravity filtered through Whatman No. 1 paper. The analytical sample was pressure-filtered through a 0.45 micron nylon filter. All samples were refrigerated subsequent to preparation, but were allowed to warm to room temperature prior to injection.

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Chromatography

The chromatographic system consisted of an M6000A solvent delivery system, a U6K injector, and an M440 ultraviolet detector (254 nm), all from Waters Associates, and a Hewlett-Packard 3390A integrator-recorder for measurement of retention times with the various mobile phases and column types. Absorbance ratios were determined using a variable wavelength system consisting of a Hitachi model 100-40 spectrophotometer fitted with an Altex flow cell. Columns used were Waters Microbondapak C_{18} and Novapak C_{18}, and a Hamilton PRP-1 resin-based C_{18}. Mobile phases used were all phosphate buffers which

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Table 1

Retention Times for Standard and Samples

Column	Mobile Phase	pH	Flow Rate ml/minute	Retention Times Standard	Minutes Sample
μBondapak C_{18}	0.5% THF in 0.001 M aq. phosphate	3.0	1.0	4.70	4.71
μBondapak C_{18}	0.5% THF in 0.001 M aq. phosphate	3.5	1.0	4.54	4.56
μBondapak C_{18}	0.5% THF in 0.001 M aq. phosphate	6.0	1.0	5.91	5.99
Novapak C_{18}	0.5% THF in 0.001 M aq. phosphate	3.0	0.5	4.00	4.00
Novapak C_{18}	0.5% THF in 0.01 M aq. phosphate	3.5	0.5	4.12	4.17
Hamilton PRP-1 Resin Based C_{18}	0.05% THF in 0.001 M aq. phosphate	6.0	1.0	3.04	3.05
Hamilton PRP-1 Resin Based C_{18}	0.5% THF in 0.001 M aq. phosphate	9.0	1.0	1.46	1.47

Table 2

Absorbance Ratios for Standard and Extract

Sample	Absorbance Ratio 245/270 nm
Uridine	0.589
Defatted Cacao Extract	0.566

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varied between 0.01 and 0.001 Molar and pH 3 to 9, containing 0.05 to 0.5 percent purified tetrahydrofuran. All mobile phases were passed through a Millipore 0.45 micron filtration system and degassed prior to use.

Standards

The uridine standard (Sigma Chemical Company) was dissolved in HPLC grade water at a concentration of 0.1 mg/ml, pressure-filtered through a 0.45 micron filter, and refrigerated until use.

Analysis

Samples of the standard uridine solution and the cacao liquor extract were injected successively into the HPLC unit after the column had been equilibrated with the desired mobile phase, and the retentions times compared.

Absorbance ratios[4] were measured at 245 nm and 270 nm at pH 3.0 in a mobile phase consisting of 0.001M. phosphate containing 0.5% tetrahydrofuran at a flow rate of 0.5 ml/minute.

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Results

The data presented in Table 1 showing the retention times for both standard and sample using various columns, pH's and flow rates, and the absorbance ratios given in Table 2 support our findings that uridine is present in the extract.

CONCLUSION

HPLC methodology has shown uridine to be a minor component of defatted cacao liquor. This finding had extended the utility of the phosphate buffer - tetrahydrofuran system on reverse phase columns as a general method for the separation of xanthines, purines, and both purine and pyrimidine nucleosides through variation of pH and organic content of the mobile phase with a variety of column types.

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