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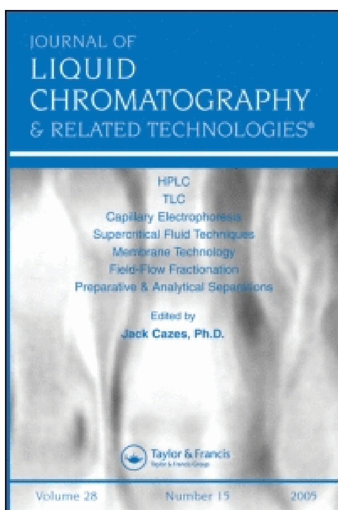

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THE IDENTIFICATION OF ADENOSINE IN CACAO PRODUCTS

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ABSTRACT

Adenosine has been identified as a component of defatted chocolate liquor through HPLC of aqueous extracts. The nucleoside was identified by comparing its behavior with an authentic sample in a variety of mobile phases, column types, and absorbance ratios at 254 and 245 nm.

INTRODUCTION

Our laboratories have previously reported the presence of 7-methylxanthine and adenine in defatted cacao liquor through HPLC methodology.[1,2] The implication of these compounds in the biogenesis of

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xanthine bases was reported as an analogous extension of Ogutuga and Northcote's investigations of similar biosynthetic systems in tea.[8] This has led us to extend our methodology to other types of compounds believed to be potential intermediates in the biosynthetic pathways of cacao, and has led to the identification of the common nucleoside, adenosine, as a minor basic component of aqueous extracts of defatted cacao liquor. Our methodology has thus been shown to be effective for identification of xanthines, purines, and nucleosides in the same type of sample.

Sample Preparation

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Finely divided chocolate liquor (10 g) obtained from the Hershey Chocolate Company, was defatted by extraction with two 75 ml portions of petroleum ether, and the residual solvent was allowed to evaporate in the hood draft. An aliquot of the sample (2.5 g) was dispersed in HPLC grade water (50.0 g), and the suspension was held at a low boil, with stirring, for 30 minutes. After cooling to room temperature, the sample was brought up to the original weight by addition of HPLC grade water and gravity filtered through Whatman No. 41 paper. An aliquot of the extract was pressure filtered through a 0.45 µm nylon filter, and used for analysis. All samples were refrigerated between analyses, but were allowed to warm to room temperature prior to injection.

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 determination of retention times in the various mobile phases. Absorbance ratios were determined using a variable wavelength Hitachi Model 100-40

spectrophotometer fitted with an Altex flow cell. Columns used were 300 x 4.0 mm Bondapak C_{18} (10 µm, Waters Associates) and Zorbax ODS (10 µm, DuPont). Mobile phases used consisted of 0.01M phosphate buffers with specific percentages of HPLC grade tetrahydrofuran.

Standards

Standard adenosine (Sigma Chemical Co.) was dissolved in HPLC water at a concentration of 0.1 mg/ml, pressure filtered through a 0.45 µm nylon filter, and refrigerated until used at room temperature.

Analysis

Samples of standard solution and liquor extract were injected successively into the HPLC unit after the column had been equilibrated with a specific mobile phase, and the retention times were compared. Absorbance ratios[4] at 245/254 nm were determined at pH 3.50 (± 0.01) using a 0.010M phosphate buffer containing 1.00% tetrahydrofuran at a flow rate of 1.0 m/minute.

Results

The data in Table 1 showing retention times for baseline resolution of standard and sample at various pH values, along with the absorbance ratios[4] shown in Table 2, indicate that the band in question is due to adenosine.

CONCLUSION

Adenosine has been shown by HPLC methodology to be a minor component of defatted cacao liquor. This, along with our previous publications, indicates

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TABLE 1

Retention Times for Standard and Samples

Column	Mobile Phase	Flow Rate ml/Minute	pH	Retention Time, Minutes	
				Standard	Sample
μBondapak C_{18}	0.2% THF in 0.01M aq. phosphate	0.2	3.27	37.9	38.8
μBondapak C_{18}	1.0% THF in 0.01M aq. phosphate	0.5	3.27	12.5	12.5
μBondapak C_{18}	1.0% THF in 0.01M aq. phosphate	1.0	3.50	15.25	15.30
Zorbax ODS	0.5% THF in 0.01M aq. phosphate	1.0	4.0	15.81	15.70

TABLE 2

Absorbance Ratios for Standard and Extract at pH 3.5

Sample	Absorbance Ratio 245/254 nm
Adenosine	0.709
Defatted cacao extract	0.703

that the phosphate buffer-tetrahydrofuran system on a reverse phase column is a rather general method for separations of xanthines, purines, and purine nucleosides through variation of pH and organic content.

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