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# AUTHENTICATION OF COCOA IN MAYA VESSELS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC TECHNIQUES

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## SUMMARY

Samples of a dry residue collected from the interiors of ceramic vessels at the Maya site of Rio Azul in northeastern Guatemala were analyzed by a variety of high-performance liquid chromatographic techniques. Archeologists at the site had strong indications that the vessels contained cocoa. Since the literature indicated that cocoa would tend to be the only Mesoamerican commodity that would contain both theobromine and caffeine, initial studies concentrated on the determination of these compounds. Reversed-phase chromatography coupled with photodiode array and mass spectrometry detection confirmed the existence of these cocoa alkoloids in several of the vessels. Amino acid and fatty acid analysis were also conducted on the residues.

## INTRODUCTION

Literature indicates that cocoa cultivation had been well established by the Aztecs in Mexico and the Maya in Central America for hundreds of years when Columbus first saw the commodity in 1502 during his fourth voyage. During that voyage, he saw a cargo of cocoa beans in a ship that was sighted off the coast of the Gulf of Honduras. In 1519, Cortes and the conquistadores were introduced to cocoa when the palaces of Montezuma were captured and large amounts of cocoa beans were found[1–3]. Cocoa was introduced to the European continent shortly after this time where its consumption grew steadily.

In 1984 at the site of Rio Azul in Guatemala, location shown in Fig. 1, excavations uncovered a burial site that was later designated as Tomb 19. The tomb contained the remains of a middle-aged man and many other artifacts including fourteen pottery vessels. One of the vessels was highly decorated with a unique lid, as can be seen in Fig. 2. Further information indicated that it contained some type of liquid substance. Hieroglyphics from the vessel can be seen in Fig. 3 and were deciphered to indicate ka-ka-w(a) or cocoa[4,5]. It is not the purpose of this paper to cover the topic of cocoa

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Fig. 1. Map indicating location of Rio Azul.

history or the status of the Rio Azul expedition since both areas have been very well covered in other publications[6–8].

A series of samples from several of the vessels were provided by archeologists at the site and analyzed by a variety of techniques. After initial analytical studies further investigations centered on the vessel seen in Fig. 2, which was given the designation as


Fig. 2. Photographs of vessels that contained samples for analysis.

Vessel 15. Archeological evidence indicated that the vessels in Tomb 19 should be assigned to the period 460–480 AD during the Early Classic Period of Mayan culture[5]. The studies that were conducted centered on the determination of theobromine and caffeine since those compounds tended to be unique to cocoa. Additional data were also obtained in the areas of fatty acid analysis, general lipid analysis and amino acid analysis.

Fig. 3. Hieroglyphic from vessel.

## EXPERIMENTAL

### *Instrumentation*

The high-performance liquid chromatography (HPLC) apparatus was assembled from commercially available components. The pumps utilized were a Model 510 solvent delivery system (Waters) and a Model 6A liquid chromatograph (Shimadzu). A number of detectors were used in various stages of the study. Initial investigations were conducted with a Model 990 photodiode array (PDA) detector (Waters) for the determination of theobromine and caffeine while confirmatory data were obtained using a Model 201 liquid chromatography–mass spectrometry (LC–MS) apparatus (Vestec). Fatty acid analysis used a Model SPD-2A UV detector at 254 nm (Shimadzu) while amino acid analysis used a Model 440 UV detector at 254 nm (Waters). Data acquisition was accomplished by the use of an NEC APC III computer and associated hardware for the PDA detector and the IBM-based Teknivent Vector One mass spectrometry workstation. Differential scanning calorimetry (DSC) was accomplished through the use of a Model 910 DSC apparatus coupled to a Model 1090 thermal analyzer (DuPont Instruments).

Due to the diversity of assays accomplished a number of columns and mobile phases were used. The particulars are outlined in Tables I and II.

### *Standards*

Standard compounds were obtained from a variety of sources. The theobromine and caffeine were repurified by sublimation. These were made up to approximate concentrations of 10 μg/ml in water and stored refrigerated at −4°C.

TABLE I
HPLC COLUMNS UTILIZED

| *Column type* | *Size* | *Manufacturer* | *Assay* |
|---|---|---|---|
| 5 μm Spherisorb ODS | 30 cm × 3.9 mm | HPLC Technol. | Theobromine and caffeine |
| Resolve $C_{18}$ | 10 cm × 8 mm | Waters | Theobromine and caffeine |
| 5 μm Spherisorb ODS | 30 cm × 3.9 mm | HPLC Technol. | Fatty acid |
| Pico-Tag | 15 cm × 3.9 mm | Waters | Amino acid |

TABLE II
MOBILE PHASES USED

| *Composition (v/v)* | *Assay* | *Detector* | *Reference* |
|---|---|---|---|
| Water–methanol–acetic acid (74:25:1) | Theobromine–caffeine | PDA | 12 |
| 0.1 *M* amonium acetate–methanol | Theobromine–caffeine | MS | – |
| Gradient from 80:20 acetonitrile–water to 100 acetonitrile | Fatty acid | UV | 8 |
| Gradient from Pico-Tag buffers A to B | Amino acid | UV | 11 |

The initial standard was used for the entire course of the study. Fatty acid standards were made up to varying concentrations in chloroform and stored at −20°C.

*Other reagents*

Fatty acid analysis was accomplished using the panacyl bromide derivative procedure that was reported earlier[9].

Amino acid analyses were accomplished using the Pico-Tag method[12].

*Sample preparation*

Samples were provided by the University of Texas at San Antonio and used as received. The samples in question not only contained some of the vessel contents but also seemed to contain some of the interior of the vessel. No attempt was made to differentiate the various components due to the small sample size available. In Table III the sample preparation techniques used for each assay are outlined. After sample preparation, all samples were analyzed using the technique of interest.

## RESULTS AND DISCUSSION

Initial studies with UV detection indicated peaks in the chromatogram of the sample extract at the same point as theobromine and caffeine. Since the occurrence of peaks at the same retention times as pure compounds is inconclusive, further studies were conducted more fully utilizing the capabilities of the PDA detector. The PDA detector will provide a three-dimensional plot of time *versus* wavelength *versus* absorbance for each injection. It additionally will allow one to obtain a UV spectrum at any portion of a peak of interest whether it be leading edge, apex or trailing edge. Fig. 4 and 5 provide a chromatogram of standard and sample extract indicating the peaks of interest at 254 and 280 nm. The PDA detector additionally allowed the determination of derivative spectra of theobromine and caffeine. First and second derivative spectra were obtained for the peaks of interest and found to agree.

A series of LC–MS studies was conducted to provide further confirmation of theobromine and caffeine. Fig. 6 illustrates the LC–MS data on the series of standard compounds. The mobile phase and column used are given in Tables I and II. The mobile phase flow-rate was 1.3 ml/min. The MS interface operating temperatures were as follows: Vaporizer, 155°C; block heater, 220°C; tip heater, 233°C; lens heater, 85°C.

TABLE III
SAMPLE PREPARATION TECHNIQUES USED

| *Sample preparation* | *Assay* | *Reference* |
|---|---|---|
| Dissolve in water and filter | Theobromine and caffeine | 12 |
| Extract with chloroform, make potassium salt and derivative | Fatty acid | 10 |
| Extract with 0.1*M* HCl and make derivative | Amino acid | 11 |

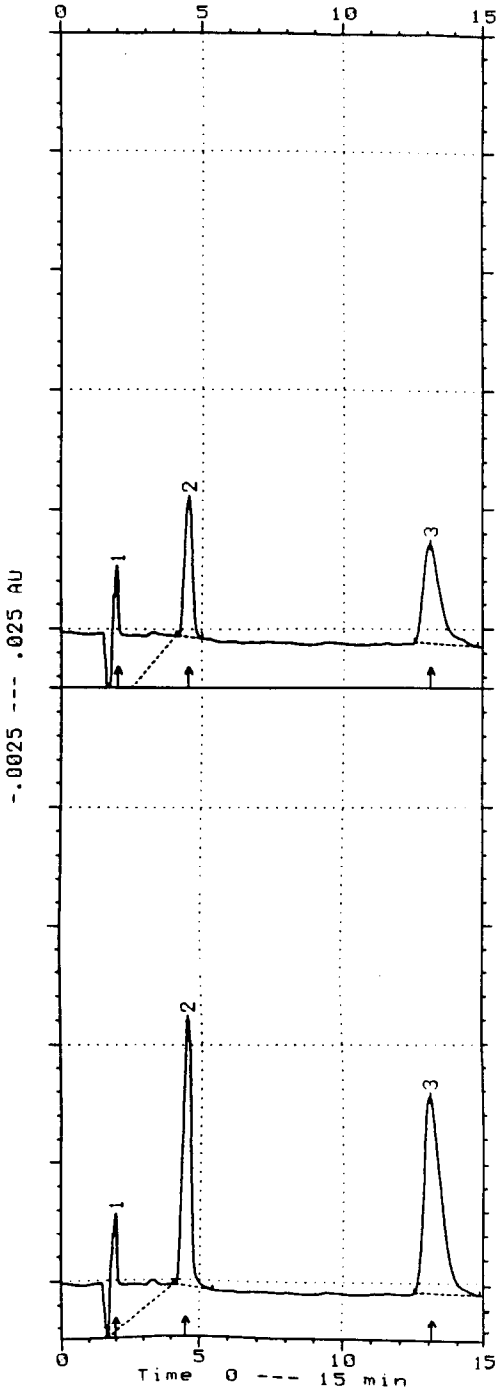


Fig. 4. Chromatograms of standard compounds. Top: 244 nm; bottom: 280 nm.

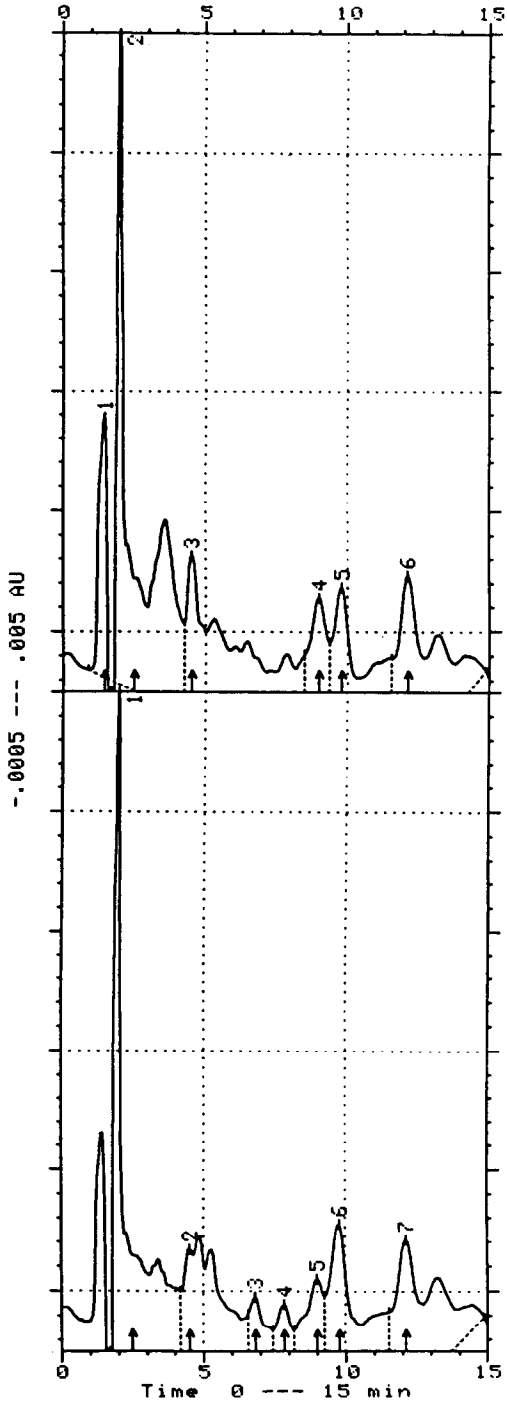


Fig. 5. Chromatograms of extract from Vessel 15. Top: 254 nm; bottom: 280 nm.

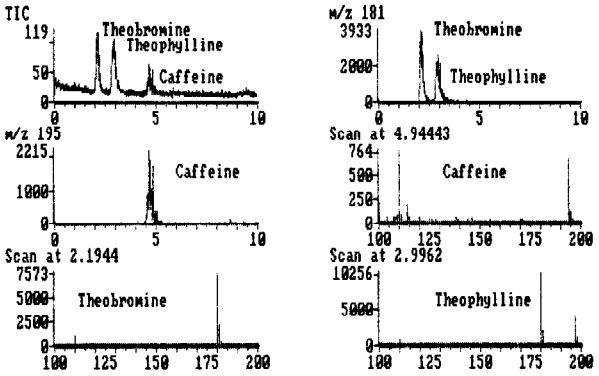


Fig. 6. LC–MS data on standard compounds.

The peaks of interest were collected and injected into the LC–MS and the extract was also injected in the "column-out" mode of operation. The total ion current (TIC) and a sample spectrum are shown in Fig. 7. Since LC–MS in the thermospray mode can be classified as a "soft-ionization" technique[13] the major ion seen is the MH+ for the compound of interest. These ions are $m/z$ 181 for theobromine and $m/z$ 195 for caffeine. A related compound, theophylline, also exhibits an $MH^+$ ion at $m/z$ 181 but does not have the same retention time as theobromine. This data provides further evidence to the existence of these compounds in this sample.

Other chemical assays were accomplished to provide further data on the contents of Vessel 15. The DSC results indicated that the vessel contained no detectable amount of lipid material. This data was further reinforced by the negative results from the fatty acid analysis. The lack of lipid is not disconcerting since even under ideal conditions one sees lipid degradation due to oxidation or other mechanisms.

An amino acid profile was also accomplished with the standards chromatogram shown in Fig. 8 and the sample chromatogram shown in Fig. 9. The results provide no information as to the existence of cocoa in the vessel but are intriguing since they do indicate the existence of some amino acids in this sample irrespective of the source.

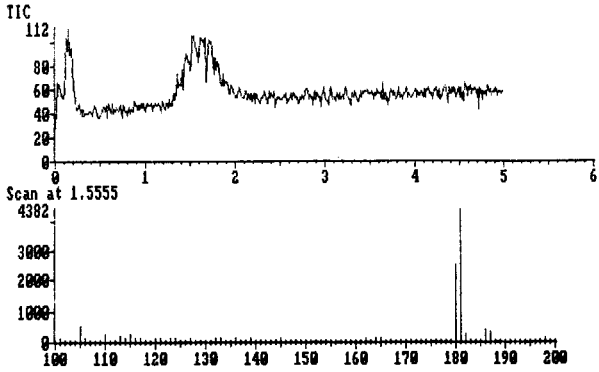


Fig. 7. Positive ion spectrum of theobromine peak from residue of Rio Azul cocoa vessel.

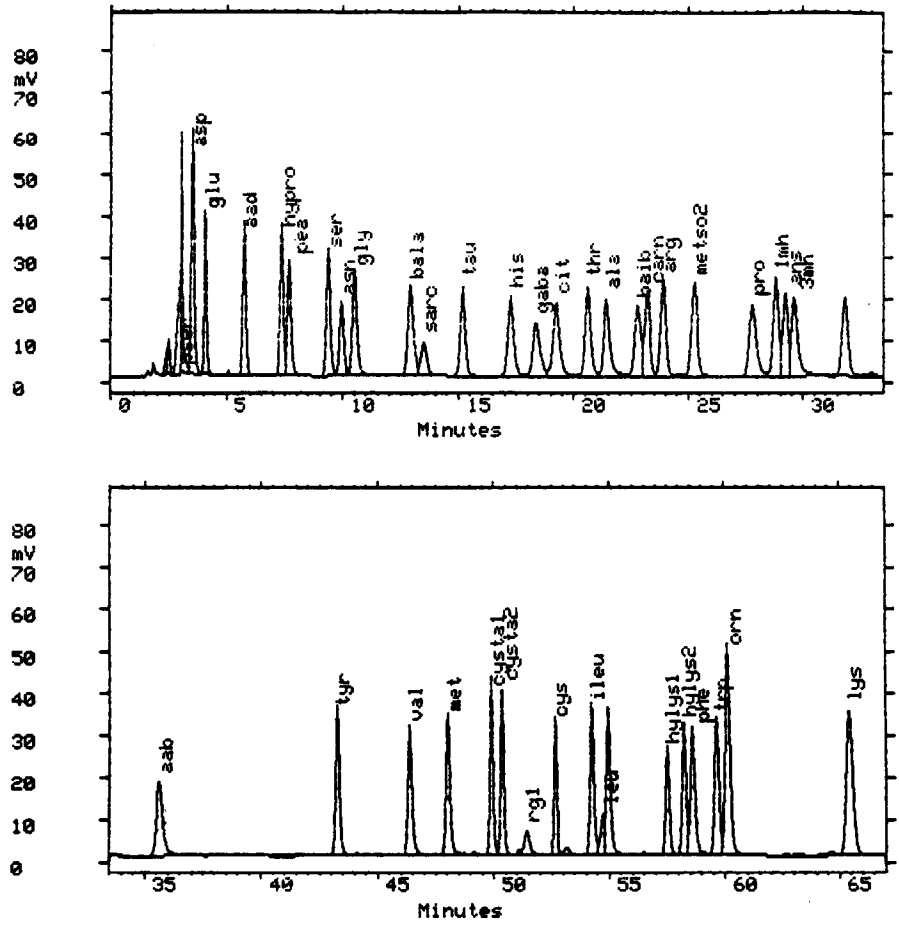


Fig. 8. Standard Pico-Tag amino acid profile.

It also is noteworthy since it provides information on the use of this assay on an archeological sample.

## CONCLUSIONS

These results provide valuable information to epigraphists on the contents of this vessel and show that it did contain cocoa in some form. For reasons of length all aspects of each assay were not outlined in detail. This study serves to illustrate the results that can be obtained from a multidisciplinary team in solving this type of problem.

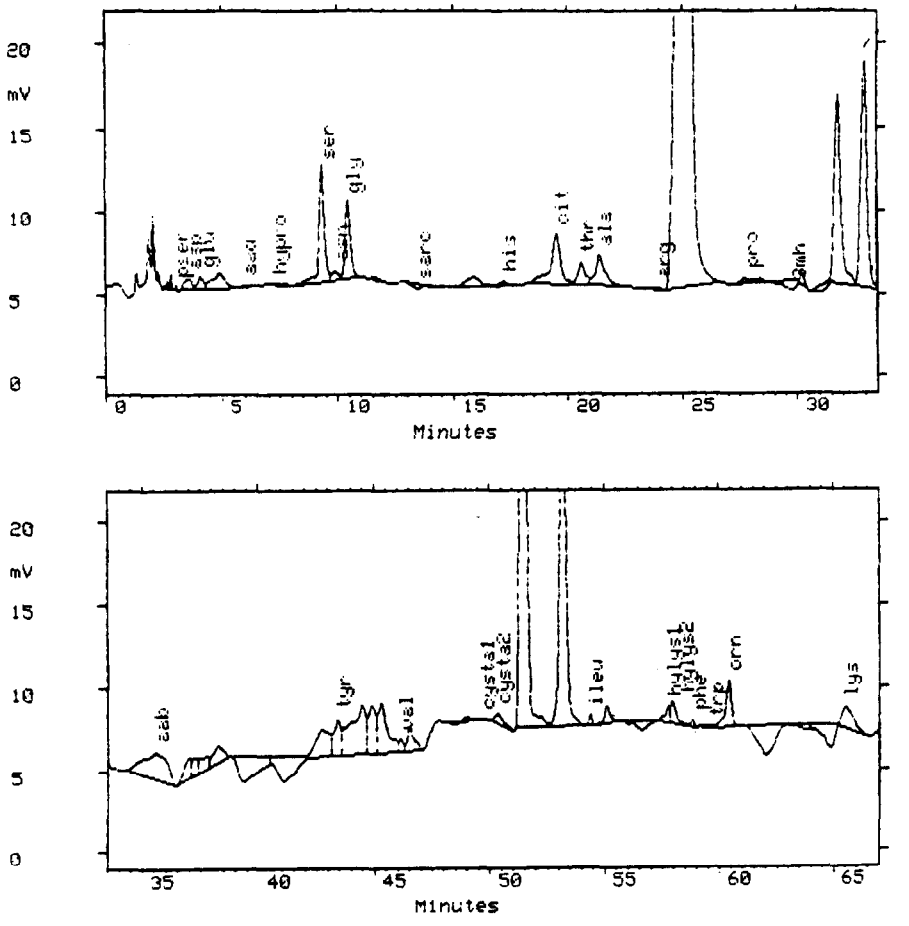


Fig. 9. Pico-tag amino acid profile of vessel extract.

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