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Betel nut residues in archaeological samples of human teeth from the Mariana Islands

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Abstract. Two tetrahydropyridine alkaloids, arecaidine and guvacine that are characteristic of betel nut (*Areca catechu* L.) have been detected in the deliberately stained labial surface of female teeth excavation on Rota, Mariana Islands. This was accomplished using selected ion monitoring techniques in conjunction with gas chromatography/electron impact-mass spectrometry. These alkaloids were not present on the buccal surface of the teeth and indicate the use of betel nut to effect the staining.
Key words. Archaeological residues; *Areca catechu* L.; betel nut; teeth; tetrahydropyridine alkaloids; arecaidine; guvacine.

The habit of chewing betel nut is widely practiced throughout India, S.E. Asia, Taiwan, Japan, the Philippines and Melanesia[1,2,3]. Its traditional usage in both India and China is well documented and it forms part of the pharmacopoeia of these civilisations where it is used for its psychoactive effects and as an anti-helminthic agent in man and animals[4,5,6]. It is thought that the use of betel in Melanesia is a similarly old practice but it has only been documented since the arrival of the Europeans in the area some 300–400 years ago. The betel nut, which is the dried endosperm from the seeds of the Areca palm *(Areca catechu)* is chewed together with lime and usually with a leaf of the betel pepper plant *(Piper betle* L.*)*. A profuse red expectorate is then produced which results in the characteristic red staining of teeth and mouth[1].

At the time of first contact with the early Spanish explorers it was a common practice amongst the Chamorro women of the Marianas to deliberately stain their teeth. The practice died out and no details of this process were recorded at the time beyond noting the use of 'blacking'[7]. Although little or no work has been done to identify the stain, it has been generally assumed that the staining was due to chewing betel nut, however, if this was indeed the case, one would expect to see staining of all the teeth[8] rather than the specific pattern of staining 'only surfaces exposed to view'[9] which is observed on mainly female teeth.

Gas chromatography/mass spectrometry (GC/MS) is a technique which is gaining increasing usage in the field of archaeology for its ability to identify organic compounds associated with archaeological artefacts[10]. Great sensitivity can be achieved and nanogram levels, or lower, can be readily detected, especially when used in conjunction with selected ion monitoring (SIM) and appropriate derivatisation. The identification of such organic compounds can be crucial in determining, for example, the former usage of potsherd[11]. Recently, we have been able to unequivocally link the use of kava, an intoxicating drink from the South Pacific, to the archaeological record by finding a set of compounds unique to the kava drink (kava lactones) in a number of archaeological artefacts[12]. We now describe a similar method for linking the use of betel nut to the archaeological record. The active principles in betel nut are the tetrahydropyridine alkaloids, arecoline, arecaidine, guvacine and guvacoline, and it is these compounds which we proposed to use as specific markers in our mass spectrometric search for residues of betel nut.

Materials and methods

Plant material. Betel nut (*Areca catechu* L.) was from Papua New Guinea. Extracts of the whole nut and of the spittal produced after mastication either with or without lime were prepared.

Archaeological artifacts. Stained teeth were obtained from an adult female burial excavated at the site of Alaguan on Rota, Mariana Islands. The burial dated to the Latte Phase (1100–1700 AD).

Sample preparation, extraction and derivatisation. Whole betel nut was ground using a mortar and pestle. Spittal samples were dried at 60 °C. Samples were homogenized with 2 ml $CHCl_3$/MeOH/H_2O (1:2:0.8, v/v/v) for 2 min and an additional 0.5 ml of $CHCl_3$ and H_2O were added sequentially followed by 1 min of ultrasonication. Samples were centrifuged and the sep-

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Table. Ions used for GC/MS detection of the betel nut alkaloids by SIM.

Alkaloid	Ions[1]	R_t (min)
Arecoline (**1**)	*m/z* 155, 140, 124, 96	2.21
TMS-arecaidine (**2**)	*m/z* 213, 198, 155, 124, 96	2.59
TMS-guvacoline (**3**)	*m/z* 213, 198, 154, 86	3.07
$(TMS)_2$-guvacine (**4**)	*m/z* 271, 256, 154, 86	3.54

[1]A total of 6 ions from either **1** and **2** or **3** and **4** were sampled in each analysis with a dwell time of 50 msec over a 0.5 u mass range.

arated $CHCl_3$ and $MeOH/H_2O$ were collected and evaporated under nitrogen.

Teeth were handled with forceps and plastic gloves. Dirt adhering to the surface was brushed off, the reddish brown stain remaining intact, and each tooth was washed with HPLC grade MeOH. Stained (labial surface) and unstained (buccal surface) areas of the teeth were ground off with a dental drill and collected directly onto aluminium foil. The powered teeth were extracted as per the betel nut samples.

Trimethylsilyl derivatives were prepared from the dried extracts (azeotroped with dichloromethane) by dissolution in BSTFA containing 1% TMCS (5 µl) and pyridine (5 µl) and heating at 90 °C for 20 min. Aliquots of 0.04–0.08 µl were injected onto the GC.

Samples and standards were run alternately on the GC/MS with blanks of BSTFA/pyridine, as used for derivatisation, to minimise the possibility of cross-contamination.

Gas chromatography/mass spectrometry (GC/MS). A fused silica capillary column (Econocap; 30 m; ID, 0.32 µm) coated with a methyl silicone bonded phase (SE-30; thickness, 0.25 µm) was eluted with He (inlet pressure 6 psi) directly into the ion source of a Finnigan 4500 GC/MS. Injections were made via a capillary on-column injector (SGE; OC1-3). The column was temperature programmed from 150 °C (hold 10 s) to 240 °C at 6 °C/min. The transfer line and interface oven were maintained at 280 °C. Electron impact (EI) mass spectra were taken at 70 eV ionisation energy and source temperature of 150 °C. Analyses were conducted using GC/MS and either full scans from 50 to 290 u in 0.3 s or SIM as recorded in the table.

Elemental analysis by electron microscopy. The teeth were examined on a JOEL JSM 6400 scanning electron microscope. The elemental analysis was by energy dispersive x-ray analysis (EDXA). The teeth were scrubbed vigorously with a toothbrush to remove adhering soil particles.

Results

Identification of betel nut alkaloids. Chloroform/methanol/water extracts of a betel nut was utilised as a standard for optimising the conditions for GC/MS. The major components and many of the minor components were identified, after trimethylsilylation, by comparison of their EIMS and GC elution times with library spectra and literature reports, as amino acids and the betel nut alkaloids (arecoline, arecaidine, guvacine and guvacoline) (figs 1 and 2). Of the four betel nut alkaloids only arecaidine and guvacine were found in spittal when betel nut was chewed either with or without lime.

It is interesting to note that the relative proportions of the betel nut alkaloids determined by us (GC peak heights: guvacine, 50; arecoline 23; arecaidine 19; guvacoline, 8) are at odds with those determined by indirect methods which would have arecoline as the major alkaloid followed by arecaidine[2]. However, it must be said that no attempt was made to optimise the extraction procedures.

Assay of alkaloids using selected ion monitoring (SIM). In order to carry out the analysis with maximum sensitivity and specificity, a number of ions were chosen from the EIMS of each of the betel nut alkaloids for

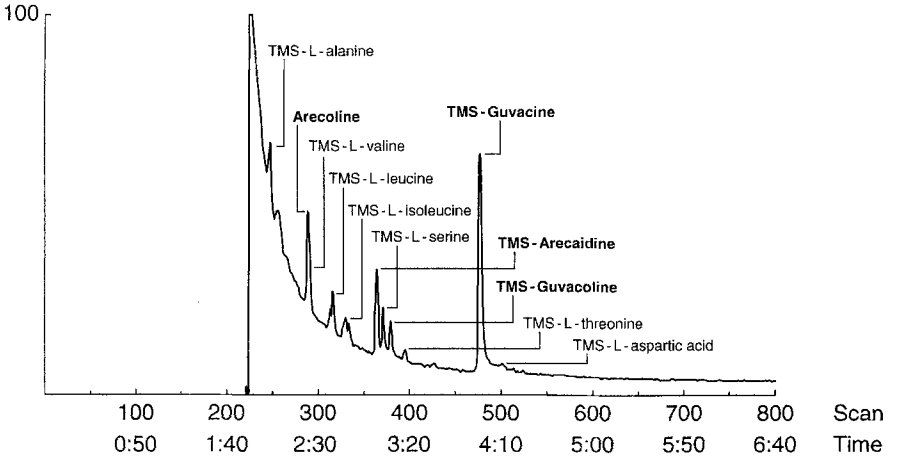

Figure 1. Total ion currents from GC/MS analysis of a $CHCl_3MeOH/H_2O$ extract of a betel nut.

selected ion monitoring (table). A number of samples from teeth were then extracted and analysed for the presence of arecoline, TMS-arecaidine, TMS-guvacoline and *di*-TMS-guvacine. TMS-arecadine and TMS-guvacine were detected in the extract of stained teeth surfaces and an example of the corresponding ion traces are shown in figure 3, confirming that this particular person was at some time exposed to betel nut. The detection of these two alkaloids corresponds with our detection of them in spittal. No betel nut residues were detected in the unstained areas of the teeth.

Elemental analysis of tooth surface. After carefully removing all adhering soil from the stained and unstained surfaces of the teeth no elements other than calcium and

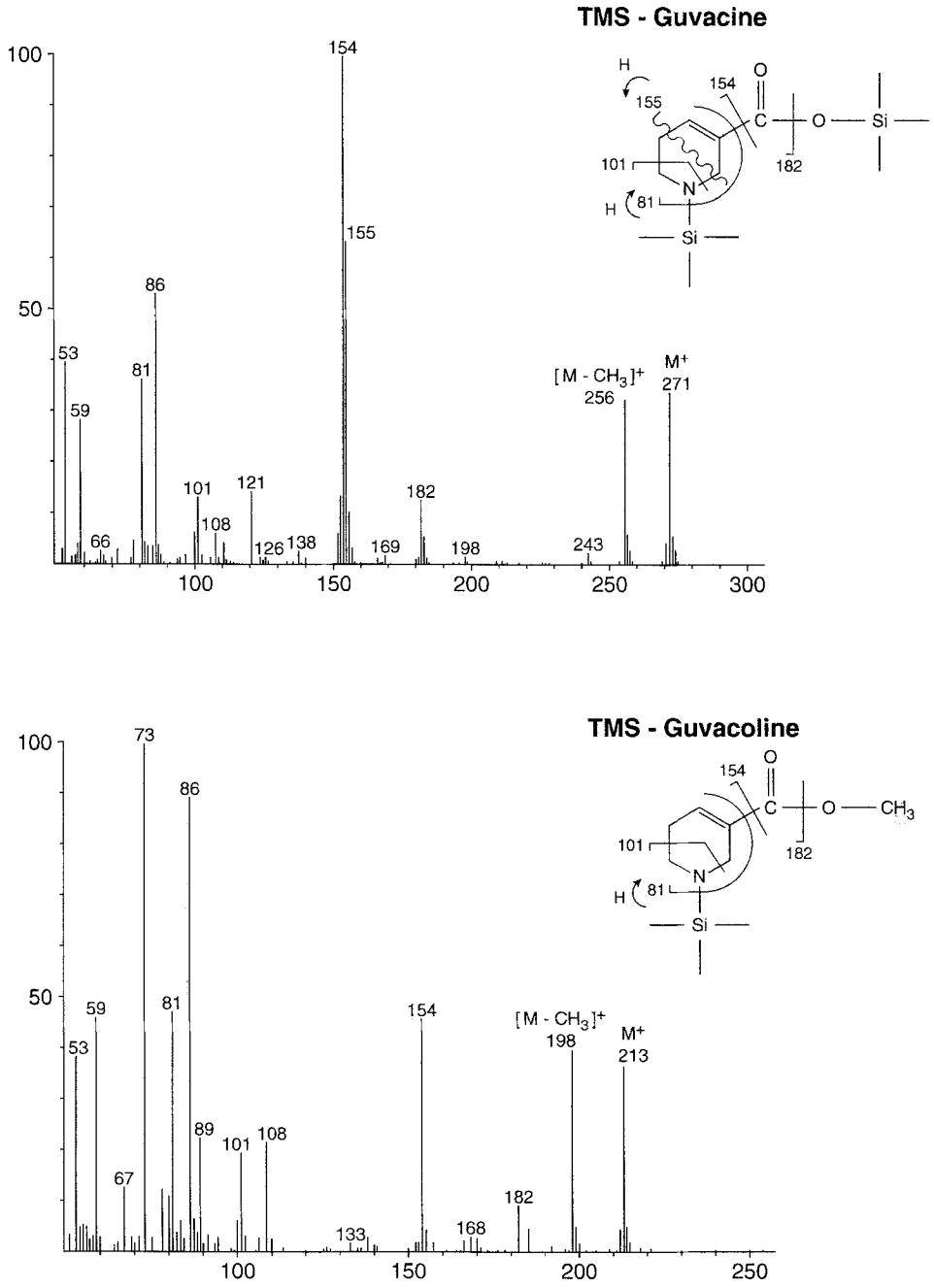

Figure 2. EI mass spectra of the betel nut alkaloids from figure 1.

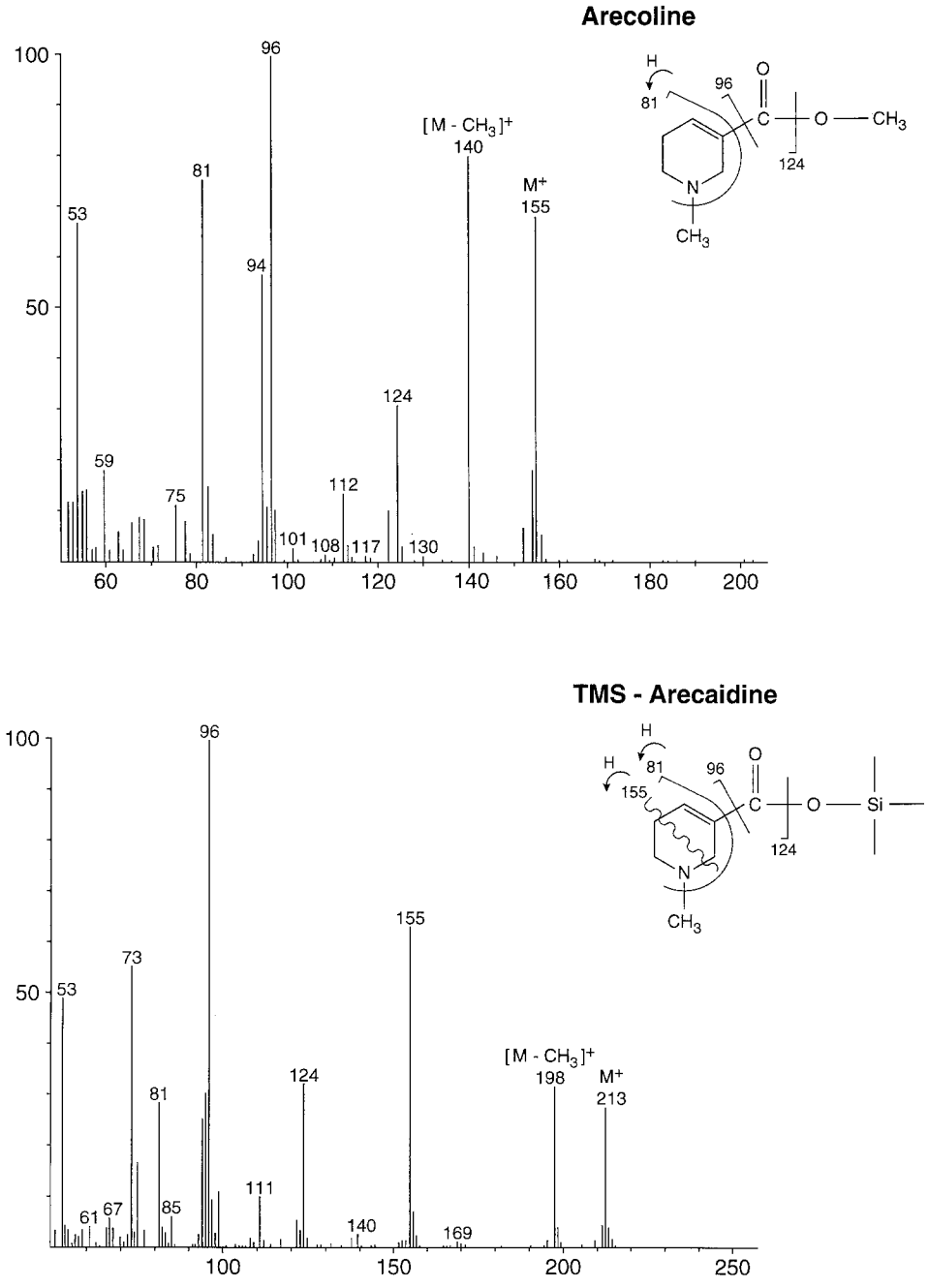

Figure 2. continued.

phosphorous were detected. The adhering soil particles contained characteristic elements aluminium, silicon and iron.

Discussion

The reddish-brown stained areas of the teeth contained two alkaloids, arecaidine and guvacine, from betel nut. These alkaloids were not detected in the unstained parts of the teeth. No clay or soil elements were found in any teeth except where adhering soil was present. These findings eliminate the use of an inorganic stain such as a magnesium-rich clay (John Craib, pers comm). The particular pattern of staining only on the visible surfaces of the teeth and the finding of betel nut residues then leads to the conclusions that, firstly, the women

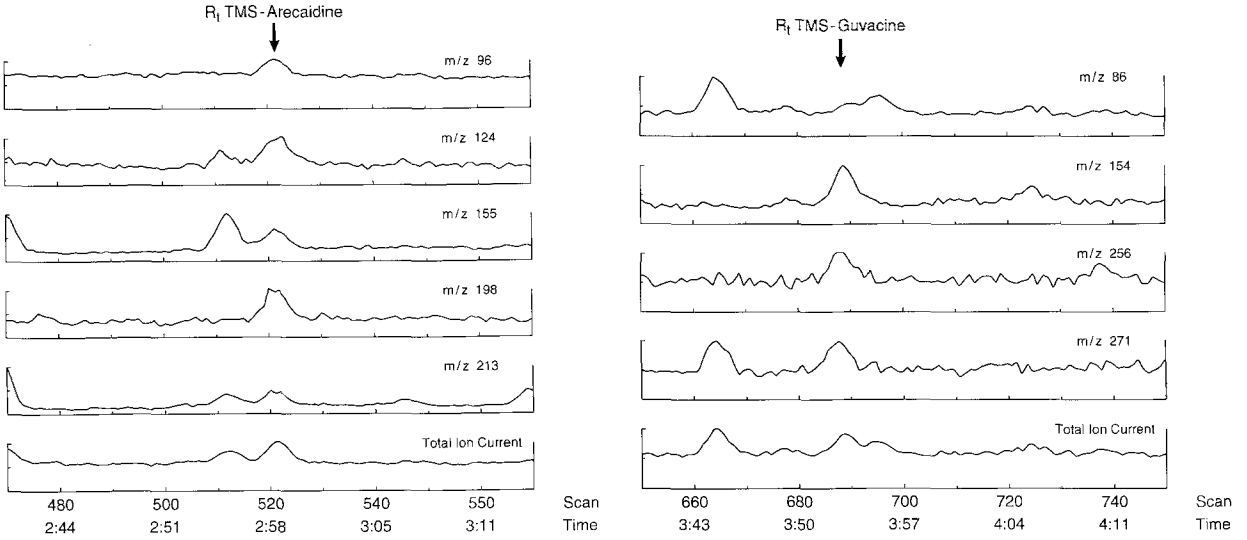

Figure 3. Arecadine and guvacine were identified in the $CHCl_3/MeOH/H_2O$ extract of stained teeth using GC/MS with SIM of the TMS derivatives. Ion current maxima were observed (see arrow) at the retention times (R_t) of TMS-arecaidine (*m/z* 96, 124, 155, 198 and 213) and TMS-guvacine (*m/z* 86, 154, 256 and 271).

did not chew betel nut on a regular basis and that, secondly, a slurry of betel nut and lime (lime produces the characteristic red colour) must have been deliberately and consistently applied to the teeth of these women. The purpose and function of this staining has been reported to be one of beautification[7,9,13].

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