Molecules from the past

Archaeology meets chemistry

Lucy Cramp and Fiona Gill

Modern archaeology uses analytical chemical techniques to answer questions about ancient civilisations and animals. Scientists working at the University of Bristol match compounds present in archaeological materials to those in modern plants and animals which were likely to have been used in ancient times. These techniques allow diverse questions to be answered. Here Lucy Cramp describes how she has identified the substances used by ancient Egyptians to preserve their dead, and on page 4, Fiona Gill reveals the likely diet of a long-extinct mammal.

A partially unwrapped mummy

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analysis
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Egyptian mummies

The Ancient Egyptians perfected the technique of preserving the bodies of their dead which was used for over three thousand years. Not only have human mummies been remarkably preserved, but also a range of animals, including cats, dogs, ibises (a type of bird), crocodiles, fish and even scarab beetles were mummified and placed into the tombs. These animals may have been pets or symbolic animals during their lifetime, or perhaps were ritual offerings to particular gods. Some animals were even placed in the tombs as joints of meat, in order to provide food for the deceased during the afterlife.

The Egyptians mummified animals as well as people – this is a mummified cat.

Many Egyptologists have tried to reconstruct the ways in which the Egyptians mummified their dead, and experimental work has even been performed (usually on rabbits and birds, rather than humans!) in order to test out some of the techniques and materials that they think the Egyptians may have been using. It is thought that after the internal organs were removed, the bodies would first have been cleaned and then dried out using natron (a naturally-occurring mixture of sodium carbonates, sodium sulphate and sodium chloride) before the body was anointed with various oils, waxes and resins and spices in order to scent the body and provide it with a waterproof coating to prevent decay. Diodorus, a Greek historian who visited Egypt in 59 BC, reported that:

> *'...they carefully dress the whole body for over 30 days, first with cedar oil and certain other preparations, and then with myrrh, cinnamon and such spices as have the faculty not only of preserving it for a long time but also of giving it a fragrant odour...'*

Chemical analyses can be performed on tiny samples of the mummy balms which allow us to identify the various substances which still remain attached to human and animal mummies from Ancient Egypt. These samples are dissolved into organic (non-water) solvents and analysed using gas chromatography and gas chromatography/mass spectrometry (see Box) in order to identify **biomarkers** for particular ingredients that may have been mixed together to make the balms. This biomarker approach involves looking for distinctive parts of molecules, called carbon skeletons, in the ancient samples which can be used as a fingerprint to compare with modern reference materials.

BOX Gas chromatography-mass spectrometry (GC-MS)

GC-MS is a powerful analytical tool for identifying molecules in archaeological and geological samples. The gas chromatograph consists of an oven containing a long, thin, silica tube through which helium gas flows continuously. Compound mixtures extracted from samples are injected at one end of the column and the oven starts to heat up slowly.

Each compound in the mixture behaves differently; some tend to stick to the stationary phase (the lining of the tube) while others remain in the mobile phase (the helium gas). This means that, as the molecules flow down the tube, the mixture starts to separate into its individual components. Usually, smaller, lighter molecules travel down the column faster than larger, heavier molecules, but the functional groups of the molecules can also affect their affinity for the stationary phase (how much they stick to it).

Each compound in the mixture reaches the end of the column at a slightly different time and passes into the mass spectrometer. In the mass spectrometer the molecules are bombarded by a stream of electrons and break into fragments. The fragments formed depend on the chemical structure of the molecule and are characteristic for each compound. They can therefore be used as a 'chemical fingerprint' to identify the molecule.

A GC-MS machine, closed and open. The chromatography takes place in the oven on the right; molecules are detected by the mass spectrometer on the left.

The analysis of the composition of mummy balms allows scientists to reconstruct the often-complex mixtures of substances that were applied to the bodies. This means that the trade routes of more exotic substances can also be reconstructed. For example, resin from conifer trees was probably imported from the eastern Mediterranean or Near East, whilst bitumen may have come from the Dead Sea. These more exotic ingredients were probably added to fats, oils and waxes that would have been locally available. Chemical analyses have revealed that whilst certain ingredients such as coniferous resin and beeswax were relatively common, there was no single formula that was in use throughout the period of mummification. This suggests that different embalmers had their own ideas about the best recipe to use!

Preparing archaeological samples for GC-MS analysis in the lab at Bristol University.

What is a biomarker?

A biomarker is a term that is used in many different fields of research, but in organic geochemistry it means a molecule of biological origin that can be directly linked to the plant, animal or microbe that produced it, on the basis of its chemical structure or stable isotopic signature. Many biomarkers are lipids, because these are common components of cell membranes and are therefore abundant in living things.

Table 1 *Analysis of samples from a number of mummies*

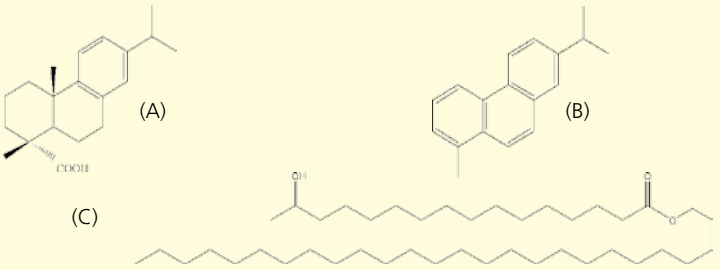
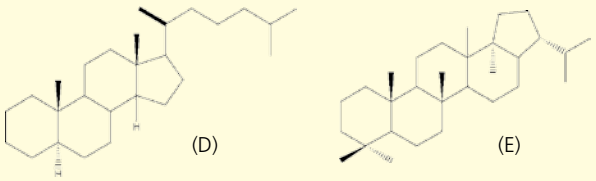
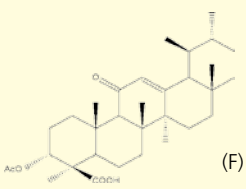
Mummy	Biomarkers present in sample
Human (female) Date: 250 BCE	
Ibis Date: 500 BCE	
Crocodile Date: 675 BCE	

Table 2 *Biomarker Guide*

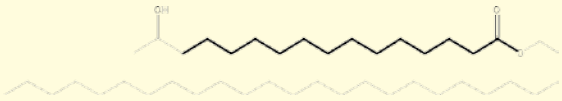
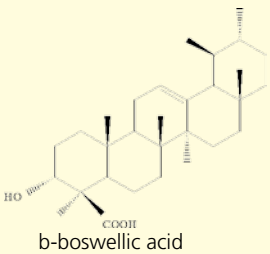
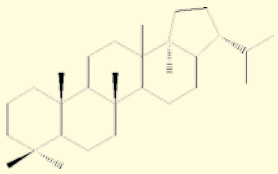
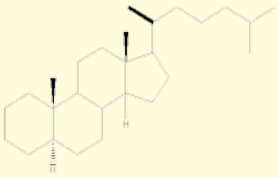
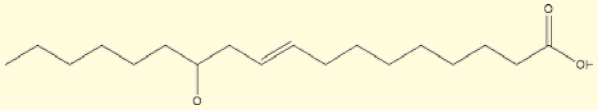
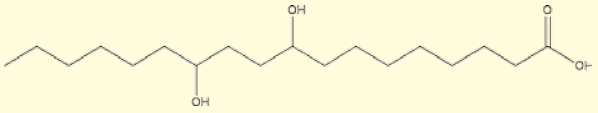
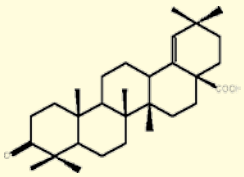
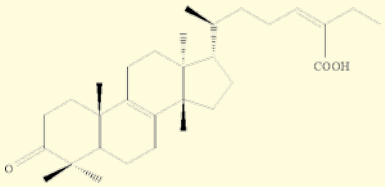
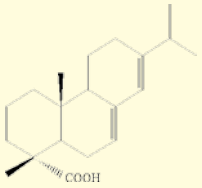
Substance	Origin	Composition	Biomarkers
Beeswax	Honeycomb	Palmitate wax esters and hydroxy wax esters; long-chain alkanes, fatty acids and alcohols	 Hexacosanyl palmitate (hydroxy wax ester)
Frankincense	Gum-resin from Boswellia shrubs growing in Arabia and parts of Africa	Plant sugars, triterpenoids (polycyclic compounds containing 30 carbon atoms)	 b-boswellic acid
Bitumen	Petroleum, derived from heat and pressure acting upon ancient organic marine matter over millions of years	Straight, branched and cyclic hydrocarbons	 17a(H), 21b(H)-hopane  a, a, a-cholestane
Castor oil	Oil from seeds of castor plant, believed to be indigenous to parts of Africa (possibly including Egypt) and the Near East	Fatty acids; mono- and dihydroxy fatty acids	 Ricinoleic acid (12-hydroxyoctadecenoic acid)  9,12-dihydroxyoctadecanoic acid
Mastic or terebinth resin	Various species of *Pistacia* shrubs, found in North Africa, Mediterranean and Near East	*Triterpenoids* including oleanonic, moronic and masticadienonic acid	 Moronic acid  Isomasticadienonic acid
Coniferous resin	Resin from pine, cedar, fir etc growing in the eastern Mediterranean and parts of the Near East	*Diterpenoids* (polycyclic compounds containing 20 carbon atoms), in particular, abietic and pimaric acids	 Abietic acid

Table 1 shows some of the diagnostic biomarkers that were discovered in three ancient Egyptian mummies. To find out which substances were used to preserve the mummies, use Table 2, the biomarker guide. Look for molecules which have a similar shape and structure to the ones in the sample. For example, biomarker C in the mummy looks like it came from beeswax. Can you work out which substances were used in each mummy? Answers on inside front cover.

What did ground sloths eat?

Imagine if, in 11 000 years time, someone could figure out what you ate for lunch today. This is what we were able to do, by looking at molecules preserved in dung from a Pleistocene ground sloth!

Alain Beneteau

A reconstruction of a ground sloth of the species Nothrotheriops shastensis – extinct for the last 10 000 years.

Dr E. Loeffler

Ancient dung – a sloth coprolite taken from Gypsum Cave, Nevada

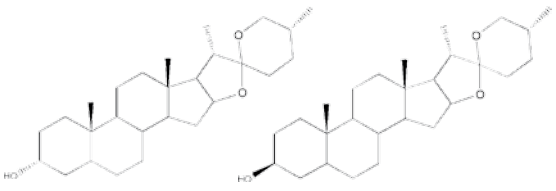
Ground sloths are bear-like creatures that lived in North and South America between 35 million and 10 000 years ago. Caves in these areas often contain dung deposits from the animals that lived in the region and because caves are cool, dry environments, the dung tends to be very well preserved. Scientists have investigated the diet of ground sloths by identifying fragments of leaves, seeds and other plant structures that survived the trip through the animal's digestive tract and are preserved in the dung. From this, and from studies of their teeth, we know that ground sloths ate only plants. However, some plants are more resistant than others to the physical and chemical processes of digestion and not all are preserved as recognisable remains in dung. Analysis of lipid (fat) biomarkers preserved in fossilised dung provides an alternative way of determining the diet of extinct animals.

Archaeologists prepare to enter Gypsum Cave, Nevada, to analyse the deposits laid down over thousands of years.

The dung that we analysed came from Gypsum Cave, Nevada, USA and was produced by a species of ground sloth called *Nothrotheriops shastensis*. Identical dung from the same locality was radiocarbon dated and found to be between 11 000 and 29 000 years old and we believe that the dung we analysed also lies within this age range. We took 1 g of the desiccated (dried) dung and ground it into a powder, extracted the organic molecules from the dung by heating it with organic solvents and separated the resulting mixture according to the chemical structure of the compounds. The sample was analysed using gas chromatography-mass spectrometry (see Box on page 2).

Environmental scientists have previously studied the organic molecules in faeces from modern domestic animals (and humans) to identify sources of faecal contamination in water supplies. We expected the organic molecules from the sloth coprolite to be similar to those found in modern herbivores such as cows or sheep, with the most abundant compounds being general plant biomarkers such as β-sitosterol (the plant equivalent of cholesterol) or 5β-stigmastanol. We did see those compounds in the sample, but they were in very low abundance. The most abundant compound was a sapogenin compound called epismilagenin. Most sapogenins are plant secondary metabolites, molecules produced by plants for non-essential functions (i.e. not growth, development or reproduction). Epismilagenin itself isn't found in plants, but is produced by the chemical alteration of another sapogenin called smilagenin. Smilagenin in dietary plants is known to be converted to epismilagenin in the digestive tract of (modern) sheep, and we believe the same thing happened in *Nothrotheriops shastensis.*

We searched published reports to compile a list of all the plant species that contain smilagenin. We then looked at reconstructions of the climate of the Gypsum Cave area and found that only two plants from the list of smilagenin-producers would have been likely to grow there at that time, namely Yucca and Agave. We therefore concluded that Yucca and/or Agave had formed a major component of the sloth's diet, at least for the few days before it produced the dung that we analysed! Future research will include analysing more dung from *Nothrotheriops shastensis* to find out whether Yucca and Agave were typical components of the diet of this species.



Epismilagenin *Smilagenin*

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