

# Beached up-but having a whale of a time!



Professor Steven Rowland & Dr Paul Sutton in the laboratory. Credit: University of Plymouth

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One of the more unusual ingredients used in perfumery and incense for centuries, is an enigmatic material known as ambergris. As but two examples of the different uses to which ambergris was put; it was deployed in pomanders to ward of plague and to perfume gloves for famous people, such as Catherine de' Medici. It has also been used as part of a recipe for ointment to anoint the kings and queens of England-including the present Queen Elizabeth II.

At Plymouth University we began to conduct research on ambergris some years ago when, following a mystery marine pollution incident which led to many sea birds being washed up as jetsam on beaches (a mystery we were able to help solve), we gained a reputation for forensic analysis of jetsam. Following the publicity, several members of the public sent us samples of waxy material they had found on beaches, often in the hope that they were not pollutant samples, but instead were the valuable natural product, ambergris.

We tested many jetsam samples sent to us, but they were all common things, like wax, or palm oil or fat. We

began to wonder what real ambergris would look like if anyone was lucky enough to find some!

On consulting the literature, we realised that much of the so-called 'information' about ambergris was confused, conflicting or often downright wrong, even in textbooks! All that we could say firmly was that ambergris was a natural product found in about one in a hundred sperm whales and, rarely, was washed up on beaches worldwide, as jetsam.

So, we went back to the drawing board: we eventually found that there was a well-documented sample of ambergris archived in a famous museum in London. The latter sample had been obtained by dissection of a dead sperm whale in the Southern Ocean back in 1947 when commercial whaling was common.

By perseverance, we got access to this sample and analysed a solvent extract by gas chromatography-mass spectrometry (GC-MS). We had to make some modifications to published analytical methods, which had already been reported not to work well, and after some time we knew that we had a reliable way of

testing genuine ambergris extracts. Our data confirmed that the museum extract contained a known, but unusual alcohol, ambrein, plus variable proportions of sterols (1). The sterols were likely from the faeces of the sperm whale, as ambergris is formed in the colon and rectum of the animal.

Colleagues, such as Dr Tony Curtis at Plymouth, and contacts of Tony from CPL Aromas, kindly sent us ex-perfumery samples of ambergris and the curator of a museum in The Netherlands sent us some ambergris dissected from a dead sperm whale beached there in 2012. Our method worked well on all these samples (1), so we tested the method further on jetsam pieces of supposed ambergris collected from beaches in New Zealand. The extracts were dominated by ambrein, with few sterols (1).

Once our work was made public, with BBC radio and TV appearances too, we were contacted by even more people who had found jetsam on beaches worldwide: some sent us samples which proved to be the real thing and many sent samples that weren't! We published some additional methods of identification



and tested more museum samples, using combined methods including not only GC-MS, but also infrared spectroscopy and nuclear magnetic resonance spectroscopy, in collaboration with colleagues from Bangor University (2).

We noticed that our growing collection of jetsam ambergris samples varied widely in colour and we wondered how old they were. So, with a friend from Bristol University, we measured the radiocarbon ages of selected jetsam pieces from all over the globe (by now we had dozens, from as far apart as Chile and Japan (3), in part thanks to contacts made for us by Ambergris Connect Ltd). One such batch was over 1000 years old and had presumably been in the sea for all of that time (3). In fact, this time span encompasses virtually the whole recorded human history of ambergris use! The ancient samples were hard and almost pure

white, whereas some very young samples were black and sticky.

We studied the effects of light on the non-odorous ambrein isolated from a 1000y old piece and showed that formation of very low amounts of odorous Ambrox™, dihydroionone and related breakdown products was rapid (4), confirming and expanding on much earlier reports (5,6).

At this point we realised that no-one in modern times had reported the odorous volatile and semi-volatile components of jetsam ambergris, even though these were well known for ambergris essence in alcohol (6). So, with an ex-colleague by now working at Leicester University, we compared these components in jetsam of different ages and locations, by solid phase microextraction GC-MS (7). We found that a simple ratio of dihydroionone to pristane varied with age in most samples and perhaps gave an indirect indication of relative ages.

So how is ambergris and the key component, ambrein, which gives rise to the odorous breakdown products such as Ambrox™, made by the sperm whale in the first place? At present this isn't really known. Japanese workers showed in the laboratory, under non-natural conditions, that particular enzymes from bacteria will convert squalene to ambrein (8) and scientists in the People's Republic of China (9) and later in Austria (10), bioengineered these enzymes into strains of algae and yeast in order to try to manufacture ambrein in vitro.

Along with a scientist at Liverpool University we decided to study the natural ambergris samples and found that both in jetsam ambergris and ambergris removed from whales in the past, the stable isotope carbon ratios of the sterols were statistically different from that of ambrein (11); this suggests the ambrein and sterols have different origins, probably indicating



Left: Ambergris found on a beach in Cornwall UK. *Credit S Rowland*

Far Left: a beach on which ambergris has been found, Islay, Inner Hebrides, Scotland. *Credit S Rowland.*

the different actions of bacteria in the whale colon. With colleagues from Cambridge and Copenhagen universities we are now using DNA profiles to study the bacteria in different ambergris samples.

Our adventitious work on this weird and rare substance has taken us around the world, from the beaches of New Zealand to those of Europe. We've made many new friends and developed many new scientific

collaborations along the way - and we're not done yet!

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