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Volatile and semi-volatile components of jetsam ambergris

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Abstract

Volatile and semi-volatile compounds account for the odours, long valued in the perfumery industry, of the natural product, ambergris. Here we demonstrate application of solid phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) to headspace analysis of the volatiles and semi-volatiles of jetsam ambergris. The samples collected in 2017/2018, ranged from a black, sticky material from New Zealand, likely recently ejected from a sperm whale, to a white solid found on a beach in Chile and radiocarbon-dated previously to be about 1000 years old. The traces of volatile/semi-volatile compounds extracted included, odorous γ -dihydroionone and odor-free pristane (2,6,10,14-tetramethylpentadecane), as the major constituents. The ratios of these to one another and to many other minor constituents, varied, depending on sample colour and age. 122 words

Keywords: Ambergris, volatiles, SPME, Sperm whale, jetsam, ambrein.

1. Introduction

Solvent extracts of ambergris, which is a natural product of the sperm whale, but which is also found as jetsam on beaches from around the globe, comprise mainly a tricyclic alcohol ambrein (I, Figure 1), plus variable proportions of faecal steroids (Lederer et al. 1946; Lederer, 1949; Baynes-Cope, 1962; Rowland and Sutton, 2017; Rowland et al. 2018 a,b). However, ambrein is odorless and the characteristic odors of ambergris long valued in perfumery (Dugan 2011; Brito et al. 2016; Serra 2013) are in fact due to oxidation products of ambrein (Ohloff et al. 1977; Ohloff 1982). The presence of the latter has been reported in ambergris tinctures (viz: aged solutions in ethanol; Anon 1976) following steam distillation (Mookerjee and Patel 1977; Awano et al. 2005) but not, as far as we are aware, in samples of jetsam ambergris. We therefore examined the headspace of samples of jetsam ambergris by solid phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) to determine the compositions of the volatile and semi-volatiles and to monitor any differences. The samples were collected recently from beaches around the globe and had a range of ages, some established by radiocarbon dating (Rowland et al. 2018 b).

2. Results and Discussion

Previous studies of the volatiles of ambergris tinctures required use of distillation techniques followed by GC or GC-MS (Mookerjee and Patel 1977; Awano et al. 2005). However, analyses of headspace components now often employ SPME techniques in place of distillation (Zhang

and Pawliszyn 1993). These SPME methods were therefore used herein to obtain samples of the headspaces above jetsam ambergris samples.

Briefly, method optimization involved testing different fibre coatings, extraction and desorption times and GC parameters. Given the value and scarcity of the samples, extraction temperature was kept at ambient to avoid denaturing. A PDMS/DVB SPME fibre was found to give optimal results in terms of capturing a representative range of the volatile components present across all samples and extracting the greatest number of compounds with a range of different functional groups. The final method allowed fast characterisation of the volatile components of ambergris compared with previous methods, requiring minimal sample quantities for testing, was non-destructive and required minimal analysis time.

Examination of the SPME extracts (headspaces) thermally de-sorbed into the injector of a gas chromatograph-mass spectrometer, of samples found on beaches from New Zealand, the U.K. and South America, are shown in Figure 1. A GC-MS total ion current (TIC) chromatogram of a corresponding blank analysis (SPME of the headspace of a blank vial) is shown in Figure S1.

Unlike the TICs of the corresponding solvent extracts of these jetsam samples, which were all dominated by ambrein (49-97%), with varying proportions of faecal steroids (Rowland et al. 2018 b), the SPME fractions contained γ -dihydroionone (II; Figure 1) or pristane (III; 2,6,10,14-tetramethylpentadecane; Figure 1) as the dominant components. These were assigned by comparison of the mass spectra with those in a NIST library (NIST 2011). SPME inherently extracts the more organoleptically important components of ambergris, which can be lost during solvent extraction and pre-concentration. Both pristane (Lederer, 1949) and γ -dihydroionone (Ohloff 1982; Schaefer 2014) have been reported previously to be components in ambergris samples of unknown provenance (Mookerjee and Patel 1977; Awano et al., 2005), where they represented $\ll 1\%$ of extracts of distillates of ethanolic tinctures. No quantitative assessment was made in this study, but no doubt they were also very minor components of the present samples. For instance, the SPME fraction of a black, sticky sample containing ~50% steroids in the solvent extract, obtained from North Island, New Zealand in June 2017, comprised mainly γ -dihydroionone (Figure 1). In contrast, the SPME fraction of the oldest sample, dated as ~1000 y old by radiocarbon techniques (Rowland et al. 2018 b), obtained from Los Lagos, Chile in April 2017, was dominated by pristane (Figure 1). Both γ -dihydroionone and an isomer (IIb), were present in about equal abundances in this sample, but both were subordinate to pristane.

Samples of measured or adjudged intermediate ages, mostly showed intermediate ratios of γ -dihydroionone to pristane (DHI/Pr) in the SPME fractions (Figure 1 and S2). For example, a sample from Brittany, France collected in September 2017 and radiocarbon dated as 'modern'

(post-1950s; Rowland et al., 2018 b) contained mainly γ -dihydroionone (DHI/Pr = 38; Figure S2), similar to that of the New Zealand sample (DHI/Pr = 16; Figure 1), whereas a radiocarbon dated 'modern' sample from County Clare, Ireland in July 2017 (Figure S2) contained a ratio DHI/Pr = 1.5, similar to that of a sample found at Widemouth, Cornwall, UK in March 2018 (Figure 1; DHI/Pr = 1.3). A sample from Grand Somalia, Somalia, collected in May 2017 which was older (radiocarbon age 219 y), was dominated by pristane (DHI/Pr = 0.4), like the 1000 y old Chilean sample (DHI/Pr = 0.3). A further sample from Somalia also collected in May 2017, with a radiocarbon age of 545 y (dated 1690-1834 AD), was an exception to this apparent trend (Figure S1; DHI/Pr = 2.5).

Other, more minor components of the SPME fractions of the above samples included compounds assigned by comparison of the mass spectra with those in a NIST library (NIST 2011), as γ -homocyclogeranyl chloride (IV, Figure 1), Ambrox® (V, Figure 1), ambreinolide (VI, Figure 1) and traces of a related unsaturated ether (Ohloff et al. 1977; Rowland et al. 2018 c). Ambrein (I) was detected in trace amounts only in the SPME fraction from the sample from Cornwall (Figure 1), along with associated artefactual ambratrienes (Figure 1) produced from dehydration in the GC injector (Governo et al. 1977; Rowland et al. 2018 a).

The odorous constituents of jetsam ambergris are thought to arise from photo-oxidation and /or autooxidation of ambrein, with scission at the trisubstituted double bond, producing essentially monocyclic and bi- or tricyclic moieties (Ohloff et al. 1977; Ohloff 1982; reviewed by Serra 2013; Rowland et al., 2018 c). The majority of the components identified herein in the SPME fractions including ambreinolide, Ambrox®, the dihydroionones and γ -homocyclogeranyl chloride, are likely explained by an origin from such natural processes.

The exception to this is pristane, which is an odorless hydrocarbon. In nature, pristane is found in abundance in Calanoid copepod zooplankton, where it derives from the transformation of the phytol side chain of the chlorophyll a in the phytoplankton diet of the animals (Blumer et al. 1963; Avignan and Blumer 1968). Since Calanoids are dominant in the plankton in many parts of the world's oceans (Mauchline 1998), and ambergris is a powerful fixative for other substances, it is possible that the pristane in these samples originates from sorption of marine planktonic oils to flotsam ambergris during the, sometimes apparently very long (Rowland et al., 2018 b), periods at sea. Such a process might explain the higher relative proportions of Pr to DHI (low DHI/Pr ratios) in several of the older samples (e.g. Chile, 1000y old, Figure 1). This complements the previous suggestion that the source of pristane is the diet of the whale

(Lederer 1949). No doubt the contribution from both these factors and others, contributes to the occurrence of pristane in ambergris.

Of the naturally-occurring odorous compounds described herein, the distributions of the jetsam ambergris volatiles were overwhelmingly dominated by γ -dihydroionone and an isomer (II, IIb, Figure 1). This contrasts with the distributions of volatiles in ambergris tinctures after laboratory aging and distillation, in which γ -dihydroionone, whilst present, was approximately as abundant as γ -coronal, α -ambrinol, sclareolide and less abundant than 8 α ,13-epoxy-14,15,16-trinorlabd-12-ene (Awano et al. 2005). These varying combinations of the natural and *in vitro* volatile products, many with different odour thresholds, no doubt account for the subtle differences in aromas of different ambergris samples and tinctures (Panten et al. 2014).

3. Conclusions

Solid phase micro extraction (SPME) and GC-MS analysis allowed a study of the naturally-occurring volatiles and semi-volatiles of jetsam ambergris samples collected from beaches around the globe. Samples ranged in appearance from a black, sticky material from New Zealand, likely recently ejected from a sperm whale, to a 1000 year old white sample from Chile. The volatile/semi-volatile compounds included odorous γ -dihydroionone and odor-free pristane (2,6,10,14-tetramethylpentadecane) as the major constituents. The ratios of these and many other minor constituents, varied considerably, depending partly on sample colour and age. The method developed herein can now be used to study the potential production of such volatiles via the oxidation of jetsam ambergris (cf Rowland et al. 2018c).

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References

Anon. 1976. Ambergris tincture. Food and Cosmetics Toxicology. Supplement, 14: 675-676.

Avignan J. Blumer M. 1968. On the origin of pristane in marine organisms. *J Lipid Res.* 9:350-352.

Awano K. Ishizaki S. Takazani O. Kitahara T. 2005. Analysis of ambergris tincture. *Flavour and fragrance journal.* 20:18-21.

Baynes-Cope AD. 1962. Analyses of samples of ambergris. *Nature* 193:978-979.

Blumer M. Mullin MM. Thomas DW. 1963. Pristane in Zooplankton. *Science* 140:974.

Brito C. Jordão VL. Pierce GJ. 2016. Ambergris as an overlooked historical marine resource: its biology and role as a global economic commodity. *Journal of the Marine Biological Association of the United Kingdom* 96:585-596.

Clarke R. 2006. The origin of ambergris. *LAJAM.* 5:7-21.

Dugan H. 2011. The ephemeral history of perfume. *Scent and sense in modern England.* John Hopkins University Press, Baltimore, USA. Chapter 5. Oiled in ambergris.

Governo TF. Alessandro RT. Pragger MJ. 1977. Gas-liquid chromatographic-mass spectrometric detection and identification of ambergris. *J AOAC Int.* 60:160-164.

Kemp C. 2012. *Floating gold : a natural (and unnatural) history of ambergris.* University of Chicago Press. 232 pp.

Lederer E. 1949. Chemistry and biochemistry of some mammalian secretions and excretions. *J Chem Soc.* 2115-2125.

Lederer E. Marx F. Mercier D. Perot G. 1946. Sur les constituants de l'ambre gris II. Ambreine et coprostanone. *Helv Chim Acta.* 29:1354-1365.

Mauchline J. 1998. *The Biology of Calanoid Copepods.* Advances in Marine Biology. 33. Academic Press.

Mookherjee B. Patel R. 1977. Isolation and identification of volatile constituents of tincture ambergris. *7th International Congress of Essential Oils.* 136:479-482.

Mori K. Tamura H. 1990. Triterpenoid total synthesis I. Synthesis of ambrein and Ambrox®. *Liebigs Ann. Chem.* 361-368.

NIST/MS. 2011. <http://webbook.nist.gov/chemistry/>. Accessed May 2018.

Ohloff G. 1982. The fragrance of ambergris. Chapter 15. Fragrance chemistry. Academic Press. 535-573.

Ohloff G. Schulte-Elte KH Müller BL. 1977. Formation of ambergris odorants from ambrein under simulated natural conditions. *Helv Chim Acta* 60:2763-2766

Oritani T. Yamashita K. Matsui M. 1970. Chemical studies of ambergris. Part IV. Configuration of ambrein. *Agri Biol Chem.* 34:1244-1248.

Panten J. Surburg H. Hölscher B. 2014. Recent results in the search for new molecules with ambergris odor. *Chemistry Biodiversity* 11:1639-1650.

Rowland SJ. Sutton PA. 2017. Chromatographic and spectral studies of jetsam and archived ambergris. *Nat Prod Res.* 15:1752-1757.

Rowland SJ. Sutton PA. Belt ST. Fitzsimmons-Thoss V. Scarlett AG. 2018a. Further spectral and chromatographic studies of ambergris. *Nat Prod Res.* [doi:10.1080/14786419.2018.1428599].

Rowland SJ. Sutton PA. Knowles TM. 2018b. The age of ambergris. *Nat Prod Res.* [doi:10.1080/14786419.2018.1523163].

Rowland SJ. Payne D. Sutton PA. 2018c. Simulated environmental photo- and autooxidation of ambrein [pre print: 10.31223/osf.io/x8d4e].

Schaefer B. 2014. Natural products in the chemical industry. Springer. 831 pp.

Serra S. 2013. An expedient preparation of enantio-enriched ambergris odorants starting from commercial ionone alpha. *Flavour Frag J.* 28:46-52.

Zhang Z. Pawliszyn J. 1993. Headspace solid-phase microextraction. *Analytical Chemistry* 65: 1843-1852.