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2024-07-01

Chemical analysis of flotsam ambergris

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Recommended Citation

Rowland, S., Sutton, P., Lewis, C., Knowles, T., Wilde, M., Alves, F., & Clough, R. (2024) 'Chemical analysis of flotsam ambergris', *Natural Product Research*, . Available at: https://doi.org/10.1080/14786419.2024.2361863
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PEARL

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Published in:
Natural Product Research

DOI:
10.1080/14786419.2024.2361863

Publication date:
2024

Document version:
Publisher's PDF, also known as Version of record

Link:
Link to publication in PEARL

Citation for published version (APA):
Rowland, S. J., Sutton, P. A., Lewis, C. A., Knowles, T., Wilde, M. J., Alves, F., & Clough, R. (2024). Chemical analysis of flotsam ambergris. *Natural Product Research*. https://doi.org/10.1080/14786419.2024.2361863

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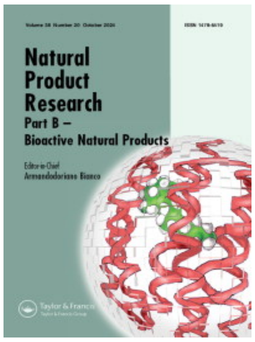
Download date: 28. Oct. 2024



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Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/gnpl20

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To cite this article: Steven J. Rowland, Paul A. Sutton, C. Anthony Lewis, Timothy Knowles, Michael J. Wilde, Filipe Alves & Robert Clough (01 Jul 2024): Chemical analysis of flotsam ambergris, Natural Product Research, DOI: 10.1080/14786419.2024.2361863

To link to this article: https://doi.org/10.1080/14786419.2024.2361863

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NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2024.2361863

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Chemical analysis of flotsam ambergris

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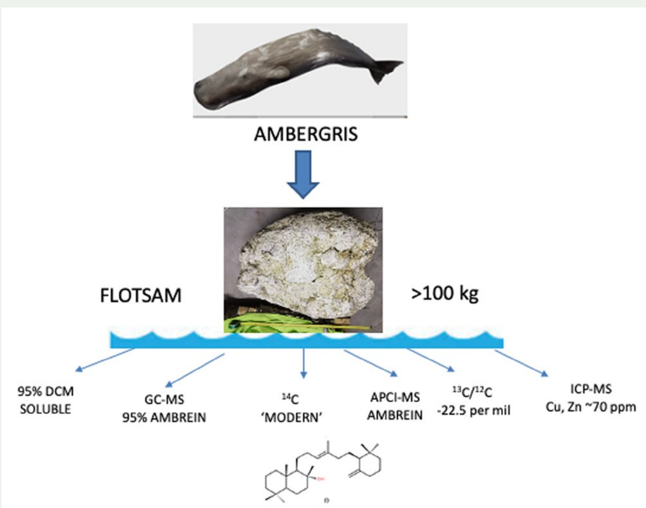
ABSTRACT

The natural product ambergris is only found rarely on beaches, as jetsam. Even more scarce, or even absent, are accounts of flotsam ambergris. Here, we report the chemical analysis of a rare, large piece (>100kg) of flotsam found in the Atlantic in 2019. About 95% of subsamples from the outside of the coprolith was soluble in dichloromethane. Of this, FTIR spectroscopy, APCI-MS and GC-MS indicated the presence of ambrein. Radiocarbon dating indicated that the sample was post 1950s in age. The $^{13}C/^{12}C$ isotope ratio (−22.5 ‰) was typical of those reported to date for whale 'body' ambergris. Metals of ambergris have hardly been reported previously. The distribution found here for the flotsam, was dominated by copper and zinc, which is similar to that of several squid species. This is also consistent with the presence of squid beaks in the coprolith. Squid are a major prey species of sperm whales.



ARTICLE HISTORY

Received 2 February 2024
Accepted 26 May 2024

KEYWORDS

Ambergris; flotsam; *Physeter macrocephalus*; atlantic; ambrein; squid; copper

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2024.2361863.

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1. Introduction

In 1954, in the journal *Nature*, Clarke reported a 'huge haul of ambergris' (Clarke 1954), which is a rare natural product of sperm whales *Physeter macrocephalus* (Cornon 1955, Clarke 2006, Mikhalev 2014). This 'body' ambergris was found inside a male whale in the Southern Ocean (Clarke 1954). Ambergris has also been rarely but consistently found as jetsam on beaches around the globe (Brito et al. 2016; Rowland et al. 2018, 2021; Wilde et al. 2020). Since it is found as jetsam, ambergris must logically also occur as flotsam, but so far as we are aware, no scientific reports of flotsam ambergris have been published.

Here, we studied the chemical composition of a floating boulder of ambergris (flotsam) retrieved from the Atlantic in 2019, which weighed over 100 kg (Figure S1). Flotsam ambergris is very rare, and this is an unusually large piece.

2. Results and discussion

Jetsam ambergris sometimes contains whole or fragments of squid beaks, which were abundant in the present flotsam sample (Figure S1). Scientific methods for the assignment of ambergris on the basis of ambrein (I) content, have been described in detail previously, including the use of radiocarbon dating (Rowland et al. 2019), Fourier transform infra-red spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS) with and without derivatisation (Governo et al. 1977; Rowland and Sutton 2017), ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (Rowland et al. 2018), isotope ratio GC-MS (Rowland et al. 2021), atmospheric pressure chemical ionisation-MS (APCI-MS; Rowland et al. 2024) and DNA profiling (Macleod et al. 2020). In the present study of flotsam, several of the above methods were used to assign the material as ambergris.

For instance, radiocarbon dating (Table S1) indicated an age post-1950s (i.e. 'modern') with an F14C value (1.0566) consistent with this (Rowland et al. 2019).

About $95 \pm 3\%$ ($n = 3$) of each of three 40-60 mg subsamples was soluble in dichloromethane (DCM). This is typical of many samples of jetsam ambergris examined previously, which also had high contents of organic-soluble material (Rowland et al. 2019). The FTIR spectra of DCM extracts contained the typical features of spectra of ambergris solutions, dominated by absorptions due to the functional groups of ambrein (I). Thus, a broad transmittance at 3372 cm^{-1} was attributed to H-bonded hydroxyl O-H stretching. A weak transmittance at ~ 3067 cm^{-1} was indicative of unsaturation and attributed to C-H stretch in an alkene. Transmittances at 2925 and 2863 cm^{-1} were attributed to C-H stretching in methyl and methylene groups and those at 1461 and 1382 cm^{-1} to the corresponding bending vibrations. Transmittances at 1644 cm^{-1} were attributed to C=C stretch and at 887 cm^{-1} to the =C-H out of plane bend (cf Governo et al. 1977; Rowland and Sutton 2017; Rowland et al. 2018).

Similarly, the ^{1}H and ^{13}C NMR spectra (Figure S2) comprised resonances indicative of the distinctive features of ambrein, including those assigned to the alkenic H and C (cf Rowland et al. 2018; Rowland et al. 2018a). For example, resonances at 4 to about 5 ppm (Figure S2a) in the ^{1}H NMR spectrum were assigned to alkenic protons. The two broad singlets occurring at 4.5 and 4.7 ppm were assigned to the two methylenic protons (Rowland et al. 2018). Similarly, the triplet at about 5.2 ppm was assigned to the vinylic proton at 3′ (Figure S2a). These characteristic resonances allow the presence of ambrein (and identity of the origin of the sample as ambergris) to be assigned. In addition, the resonances due to two methyl groups of ambrein were also assigned (Figure S2a). The presence of minor unknown constituents was indicated by other minor resonances in the region between 0.5 and ~2.2 ppm. These underlay the characteristic resonances of pure ambrein (Rowland et al. 2018). The ^{13}C NMR spectrum of the whole dichloromethane extract of the flotsam ambergris re-dissolved in deuterated chloroform, is shown in Figure S2b. Resonances at ~100-150 ppm were assigned to alkenic carbon atoms by comparison with published spectra (Rowland et al. 2018). The resonances occurring in this region were each assigned to C6″, 4′, 3′ and the methylenic C6″=CH_2. In addition, the resonance due to C2 was also assigned (Figure S2b).

GC-MS indicated an average composition of 95 ± 0.5% (n = 3; Table S2) ambrein, measured and identified as the TMS ether (Figure S3; cf Rowland and Sutton 2017; Rowland et al. 2018; Rowland et al. 2018a). Thus, a small ion *m/z* 485 was assigned to the $M^{+\cdot}$-CH_3 ion of the TMS ether (Rowland and Sutton 2017), whilst a significant ion at *m*/z 143 was attributed to a mono-unsaturated, C_4H_6-OTMS moiety (Rowland and Sutton 2017).

Positive ion APCI-MS (Figure S4; cf Rowland et al. 2024) also confirmed the presence of ambrein. The spectrum was characterised by a base peak ion, *m/z* 411, shown previously by high resolution accurate mass APCI-MS to originate from the protonated molecular ion (*m/z* 429; $[MH]^+$) of the hindered alcohol ambrein (I) by water loss (Rowland et al. 2024).

The approximate $^{13}C/^{12}C$ isotope ratio was −22.5 ‰ (Table S1). (Repeat analyses established previously that the reproducibility of the latter method was ± 0.4 ‰; Rowland et al. 2019). For comparison, the stable isotopic compositions of the carbon in jetsam ambergris from both hemispheres examined previously had a mean value of −21.6 ± 1.9 ‰; n = 26: Rowland et al. 2019). The stable isotope ratios of blubber, skin, liver and muscle from seven northern hemisphere sperm whales from the Adriatic coast of Southern Italy was −18.5 ± 1.2 ‰ (Mazzariol et al. 2011).

ICP-MS revealed the relative and absolute concentrations of metals in the flotsam (Figure S5). Lederer (1950) postulated that the metals in ambergris might reflect the metal composition of the squid diet of the whale, particularly the squid haemolymph, in which copper complexed as haemocyanin, would be the major metal. Ohloff et al. (1977) suggested that copper porphyrins in ambergris would act as catalysts for photo- and auto-oxidation of ambrein; photo-oxidation experiments with 5, 10, 15, 20-tetraphenyl-21H, 23H-porphine copper (II), supported this (Rowland and Payne 2018). Few data for metal contents of ambergris have been published to date.

Copper was the major element detected in the flotsam ambergris studied herein (~70 µg g^{-1} dried weight in the solid; Figure S5), but concentrations of zinc were similar (~50 µg g^{-1}), whilst iron and cadmium were present at appreciable, but somewhat lower, concentrations (~20 µg g^{-1}). Although ambergris is heterogenous (Baynes-Cope 1962), the metal contents of triplicate samples were quite reproducible herein (Figure S5). This metal profile suggests to us that the metal content of the flotsam reflects that of the liver, mantle, eyes and haemolymph of the squid in the sperm whale diet, rather than that of squid haemolymph alone (cf Lederer 1950).

Examination of a wider collection of samples (e.g. those studied by Rowland et al. 2019), will now allow this theory to be tested further.

3. Experimental

The experimental details are provided in the Supplementary Material.

4. Conclusions

Analysis by multiple methods of a large piece of rare flotsam ambergris found in the Atlantic in 2019 produced data consistent with a post 1950s origin from a sperm whale. The metals profile was similar to that of the 'whole' body (e.g. liver, mantle, eyes and haemolymph) of the squid in the sperm whale diet, rather than that of squid haemolymph alone.

Acknowledgments

We thank J. Quinn, University of Plymouth, for help with production of the graphical abstract (photos in graphical abstract: E.Berninsone/ARDITI and F. Alves).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The Portuguese Foundation for Science and Technology (FCT) supported FA throughout the strategic projects UIDB/04292/2020 awarded to MARE and LA/P/0069/2020 granted to the Associate Laboratory ARNET.

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