



# Formaldehyde from marine algae

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## Abstract

Formaldehyde, as its dimedone adduct (formaldemethone), has been isolated and fully characterized from the marine algae *Ulva lactuca*, *Codium fragile* ssp. *tomentosoides* (Chlorophyta) and *Palmaria palmata* (Rhodophyta). Formaldemethone has also been detected by TLC (thin-layer chromatography) and quantitatively estimated by OPLC (overpressured layer chromatography) in extracts of all the other species of seaweeds tested, which included representatives of the green, red and brown algae. It was concluded that formaldehyde is probably a constituent of all marine algal species. © 1998 Elsevier Science Ltd. All rights reserved.

*Keywords:* marine algae; Rhodophyta; Chlorophyta; Phaeophyta; formaldehyde; formaldemethone

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## 1. Introduction

Formaldehyde has been isolated as its dimedone adduct (formaldemethone) from both fresh and dry *Ascophyllum nodosum* (Tyihák et al., 1996). It was postulated that during methylation and demethylation processes in the cells, hydroxymethyl groups may be formed, which dependent on pH, are in equilibrium with either free formaldehyde or ions, such as iminium, oxonium and thionium. These react with dimedone to form formaldemethone (Tyihák et al., 1996). The question is raised as to whether formaldehyde is present in other marine algae and, to answer this, further brown (Phaeophyta) as well as green (Chlorophyta) and red (Rhodophyta) algae have been tested.

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## 2. Materials and methods

The algal species studied, their places and dates of collection and their nomenclatural authorities are given in Table 1. The plant material was carefully sorted to reduce contamination by extraneous material, and observed epibionts were removed by scraping with a knife. As soon as possible after collection, part of each sample was frozen in liquid nitrogen and the rest was dried in a circulating air oven at 50°C for 6 h.

### 2.1. Isolation and characterization of formaldemethone

Fresh *Palmaria palmata*, *Ulva lactuca* and *Codium fragile* ssp. *tomentosoides* (500 g), frozen and powdered in liquid nitrogen, were separately mixed with 1 l of a 2 mM solution of dimedone in methanol. After standing for 24 h, each suspension was centrifuged at 1500 g for 10 min. The clear supernatants were concentrated to dryness under reduced pressure. The residues were dissolved in chloroform and subjected to centrifugal thin-layer chromatography (centrifugal TLC) using silica gel (Merck grade 7749, TLC grade with fluorescent indicator) layers (1 mm) and chloroform as the development solvent. When examined under UV light at 254 nm, a distinct blue band was observed, which was eluted from the silica with chloroform. The major component in the eluate was further purified by preparative TLC using silica gel (Merck grade 7749, TLC grade with fluorescent indicator) layers (500 µm) and chloroform as the development solvent. The blue band seen under UV light was scraped from the plate, the compound eluted with chloroform and characterized from proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopic and electron impact (EI) mass spectrometric data. <sup>1</sup>H NMR spectra were obtained in deuterated chloroform using a Jeol GSX 270 FT-NMR spectrometer. The EI mass spectra were recorded using a Jeol DX 303 spectrometer coupled to a DA 5000 data system.

### 2.2. Detection and quantification of formaldemethone by thin-layer and overpressured layer chromatography

Samples of frozen, powdered, fresh algal tissue (500 mg) and powdered, dry plant material (250 mg) were mixed with 1.0 ml and 0.75 ml, respectively, of 0.2% dimedone solution in methanol. After standing for 24 h, the suspensions were centrifuged at 1500 g for 10 min and the clear supernatants separated for examination first by TLC and secondly by overpressured layer chromatography (OPLC) (Ferenczi-Fodor et al., 1991). TLC was performed on silica gel (Merck grade 7749, TLC grade with fluorescent indicator) layers (250 µm) with chloroform as the development solvent. Formaldemethone was detected under UV light as a blue spot on a green background.

Off-line OPLC separations were carried out with a Chrompres 25 OPLC chromatograph (Laboratory Instruments Co. Ltd, Budapest, Hungary). Algal extracts were applied with a Hamilton syringe to silica gel 60 F<sub>254</sub> chromatoplates, with impregnated edges, and developed with chloroform-methylene chloride (35:65 v/v). For the separation of residual dimedone, the chromatoplates were developed again with acetone. The presence of formaldemethone was determined by scanning the

Table 1  
Formaldehyde (as formaldehyde) concentrations in fresh samples of marine algae

Species	Place of collection	Date of collection	Formaldehyde (as formaldehyde) ( $\mu\text{g g}^{-1}$ fresh tissue)
<b>CHLOROPHYTA</b>			
Ulvothyceae			
<i>Enteromorpha intestinalis</i> (L.) Link	Kimmeridge, Dorset	July 1995	138
<i>Ulva lactuca</i> L.	Langstone Harbour, Hants	April 1997	147
Cladophoraceae			
<i>Chaetomorpha capillaris</i> (Kütz.) Berg.	Finavarra, Co. Clare, Ireland	April 1997	218
<i>Cladophora rupestris</i> (L.) Kütz.	Kimmeridge, Dorset	July 1995	13
Codiaceae			
<i>Codium fragile</i> (Sur.) Hariot ssp. <i>tomentosoides</i> (Goor) Silva	Kimmeridge, Dorset	July 1995	102
<b>PHAEOPHYTA</b>			
Phaeophyceae			
Ectocarpaceae			
<i>Pilayella littoralis</i> (L.) Kjellm.	Kimmeridge, Dorset	March 1997	72
Scytosiphonaceae			
<i>Scytosiphon lomentaria</i> (Lyngb.) Link	Kimmeridge, Dorset	April 1997	273
Desmarestiaceae			
<i>Desmarestia aculeata</i> (L.) Lamour.	Hayling Island, Hants	March 1997	+
Chordaceae			
<i>Chorda filum</i> (L.) Stackh.	Kimmeridge, Dorset	July 1995	14
Laminariaceae			
<i>Laminaria digitata</i> (Huds.) Lamour.	Kimmeridge, Dorset	July 1995	14
<i>L. saccharina</i> (L.) Lamour	Kimmeridge, Dorset	July 1995	91
	St Malo, France	February 1997	5
	Kimmeridge, Dorset	July 1995	51
	Hayling Island, Hants	March 1997	5
	Kimmeridge, Dorset	July 1995	258
<i>Saccorhiza polyschides</i> (Lightf.) Batt.			
Fuaceae			
<i>Ascophyllum nodosum</i> (L.) Le Jol.	Bull Bay, Anglesey	July 1996	23
<i>Fucus serratus</i> L.	Kimmeridge, Dorset	July 1995	17
<i>F. spiralis</i> L.	Kimmeridge, Dorset	April 1997	152
<i>F. vesiculosus</i> L.	St Malo, France	February 1997	129

—continued

Table 1  
—continued

Species	Place of collection	Date of collection	Formaldehyde (as formaldehyde) ( $\mu\text{g g}^{-1}$ fresh tissue)
<b>Himantaliaceae</b>			
<i>Himantalia elongata</i> (L.) S.F. Gray	Hayling Island, Hants	March 1997	87
<b>Cystoseiraceae</b>			
<i>Cystoseira tamariscifolia</i> (Huds.) Papenf.	Kimmeridge, Dorset	July 1995	4
	Kimmeridge, Dorset	March 1997	27
	Kimmeridge, Dorset	March 1997	148
	St Malo, France	February 1997	115
<i>C. baccata</i> (S. Gmel.) Silva	Kimmeridge, Dorset	July 1995	29
<i>Halidrys siliquosa</i> (L.) Lyngb.	Hayling Island, Hants	March 1997	83
<b>Sargassaceae</b>			
<i>Sargassum muticum</i> (Yendo) Fensholt	Kimmeridge, Dorset	July 1995	29
	Hayling Island, Hants	March 1997	83
<b>RHODOPHYTA</b>			
<b>Florideophyceae</b>			
<b>Furcellariaceae</b>			
<i>Furcellaria lumbricalis</i> (Huds.) Lamour.	Kimmeridge, Dorset	July 1997	145
<b>Rhodophyllidaceae</b>			
<i>Calliblepharis jubata</i> (Good. et Woodw.) Kütz.	Kimmeridge, Dorset	March 1997	51
<b>Phylloporaceae</b>			
<i>Phyllophora crispa</i> (Huds.) Dixon	Kimmeridge, Dorset	April 1997	453
<b>Gigartinaeae</b>			
<i>Chondrus crispus</i> Stackh.	Kimmeridge, Dorset	July 1995	44
<i>Mastocarpus stellatus</i> (Stackh.) Guiry	Bembridge, Isle of Wight	November 1994	289
<b>Corallinaceae</b>			
<i>Corallina officinalis</i> L.	Kimmeridge, Dorset	March 1997	15
<i>Jania rubens</i> (L.) Lamour.	Kimmeridge, Dorset	April 1997	11
<b>Dumontiaceae</b>			
<i>Dilsea carnosa</i> (Schmidl) O. Kuntze	Kimmeridge, Dorset	March 1997	99
<i>Dumontia incrassata</i> (O.F. Müll.) Lamour.	Kimmeridge, Dorset	March 1997	81
<b>Palmariaaceae</b>			
<i>Palmaria palmata</i> (L.) O. Kuntze	Southsea, Hants	February 1996	151
<b>Champiaceae</b>			
<i>Chylocladia verticillata</i> (Light.) Bliding	Kimmeridge, Dorset	July 1995	285

<i>Gastroclonium osatum</i> (Huds.) Papenf	Kimmeridge, Dorset	March 1997	111
<i>Lomentaria articulata</i> (Huds.) Lyngb.	Kimmeridge, Dorset	March 1997	220
Ceramiales			
<i>Ceramium rubrum</i> (Huds.) C. Ag.	Kimmeridge, Dorset	July 1995	241
Rhodomelales			
<i>Halopitys incurvus</i> (Huds.) Batt.	Kimmeridge, Dorset	March 1997	260
<i>Osmundea hybrida</i> (A.P. de Candolle) Nam	Kimmeridge, Dorset	April 1997	280
<i>O. pinnatifida</i> (Huds.) Stackh.	Kimmeridge, Dorset	March 1997	82
<i>Polysiphonia lanosa</i> (L.) Tandy	Kimmeridge, Dorset	July 1995	757
	Kimmeridge, Dorset	March 1997	135
Bangiophyceae			
Bangiaceae			
<i>Porphyra linearis</i> Grev.	Southsea, Hants	February 1997	586
<i>P. leucosticta</i> Thur.	Kimmeridge, Dorset	March 1997	135

\*Detected by TLC only.

All the places of collection are in the U.K., unless otherwise stated.

developed chromatograms with a Shimadzu CS 930 scanner at 260 nm (Gersbeck et al., 1989) and comparing the absorbance intensities with those produced by known concentrations of formaldehyde (Tyihák et al., 1996).

### 3. Results and discussion

When fresh *Ulva lactuca*, frozen and powdered in liquid nitrogen, was mixed with dimedone solution, a compound was formed which was isolated and purified by centrifugal and preparative TLC. The compound had identical TLC, OPLC,  $^1\text{H}$  NMR spectroscopic and EI mass spectrometric characteristics to formaldehyde (Tyihák et al., 1996). Formaldehyde was also isolated when dry *Ulva lactuca* and fresh *Palmaria palmata* and *Codium fragile* ssp. *tomentosoides* were treated in the same way.

Fresh and dried samples of 41 marine algal species were tested for the presence of formaldehyde, as its dimedone adduct, using TLC. This compound was detected in the extracts of all the species analysed. However, extracts of the algae prepared with methanol alone did not show the presence of compounds with the chromatographic characteristics of formaldehyde. The quantity of formaldehyde present in the fresh samples was estimated by OPLC (Table 1). It was considered that drying the algae may lead to the alteration of various constituents and the values obtained for the quantity of formaldehyde present in the dry samples may be misleading. For this reason, only the results obtained for fresh material are quoted, even though formaldehyde was also detected in the extracts of all the dried algae tested.

The formaldehyde content recorded varied considerably from species to species and between different collections of the same species. The highest yield was from *Polysiphonia lanosa* collected in July ( $757 \mu\text{g g}^{-1}$ ), whereas another sample collected in March had a much lower content ( $135 \mu\text{g g}^{-1}$ ). This difference could be due to plant to plant variation, seasonal differences or to differences in moisture content of the plant material. The water content of algal material is variable depending on whether it has been collected from the sea or at low tide after the plant has been subjected to a certain level of dehydration. Noticeable variations were also recorded for the other species for which more than one collection was made (*Laminaria digitata*, *L. saccharina*, *Sargassum muticum* and *Cystoseira tamariscifolia*) (Table 1).

Tyihák (1987) and Tyihák et al. (1993) have postulated that the methyl group of L-methionine is formed via formaldehyde and that formaldehyde from SAM is linked to different enzymic transmethylation reactions (Husztí and Tyihák, 1986; Tyihák, 1987; Tyihák et al., 1993). Rapid formaldehyde pathways in different tissues exist through hydroxymethyl groups linked to various acceptor molecules (Tyihák et al., 1994). From this it follows that formaldehyde should be present in biological systems in detectable amounts. The results reported in this communication demonstrate that this is true for marine algae.

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