Method for photooxidation products

Sample collection

Samples of seawater for isoprenoid compound analysis were collected with the 12L Niskin bottles of the bottle rosette sampler, at four depths within the 5-150m water column. Water samples were kept in the dark at 4°C prior to filtration, which was started the day following sampling (less than 24h later). Volumes of between 20 to 32L were filtered on to GF/F Whatman glass-fibre filters for each sampled depth. Samples for GC-EIMS analyses were stored frozen (-20°C) until analyzed, generally within several weeks. In order to provide further information on the downward fluxes of the isoprenoid compounds, samples of freeze-dried particles collected in 1990 at 200m and 1000m with sediment traps (Technicap model PPS3) moored at the DYFAMED station, were also analyzed.

Treatment of samples for isoprenoids analysis

Samples (filters and freeze-dried sediment trap particles) were saponified (5% KOH in 50% methanol, reflux for 2h or 4h for sediment trap particles) and then extracted three times with hexane. The combined hexane extracts were dried over Na_2SO_4, filtered (Whatman qualitative filters) and concentrated by means of rotary evaporation. The total extract was taken up in 300 µl of a mixture of pyridine and N,O-bis-(trimethylsilyl)-trifluoroacetanamide (BSTFA from SUPELCO) (2:1, v/v) and allowed to silylate at 50°C for 2 hours. Following evaporation to dryness under nitrogen, the residue was taken up in ethyl acetate and analyzed. In order to exclude photochemical artifacts, all the treatments were carried out under dim light using aluminium foil-wrapped vessels. Blanks were systematically carried out before the analysis of the samples.

Identification and quantification of isoprenoids by GC/EIMS

Phytol and 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol were identified by comparison of their retention times and their mass spectra with those of standards[1]. Quantitative determinations were based on integrator data, which was calibrated with external standards. For low concentrations, identification and quantification were assessed by Selected Ion Monitoring (SIM) (Fig. 2) with the diagnostic ions at m/z 143 for silylated phytol and at m/z 109+143+147+353+456 for silylated 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (Rontani *et al*., 1996). GC/EIMS analyses were carried out with a Hewlett Packard 5890 series II plus gas chromatograph equipped with an on-column injector and coupled to a Hewlett Packard 5972 mass spectrometer operating at 70eV with an SGE AF516 electron multiplier over the mass range m/z 50-550 and with a cycle time of 1.5s. The gas chromatograph was equipped with a fused silica capillary column (15m x 0.25mm i.d.) coated with SGE-BPX35 (film thickness = 0.25 µm). Helium was used as the carrier gas (0.37 bar). A detailed description of the method is given in Cuny *et al.* (1999). Accuracy is estimated to be ± 0.002 nmol.l^{-1} with a detection limit of about 0.002 nmol.l^{-1}.

Cuny, P., Romano, J.C., Beker, B., Rontani, J.F., 1999. Comparison of the photodegradation rates of chlorophyll chlorin ring and phytol side chain in phytodetritus: is the phytyldiol versus phytol ratio (CPPI) a new biogeochemical index?. Journal of Experimental Marine Biology and Ecology 237, 271-290.

Rontani, J.F., Raphel, D., Cuny, P., 1996. Early diagenesis of intact and photooxidized chlorophyll phytyl chain in a recent temperate sediment. Organic Geochemistry 24, 825-832.

Cuny P., Marty J.C., Chiavérini J., Vescovali I., Raphel D. and Rontani J.F. 2002. One-year seasonal survey of the chlorophyll photodegradation process in the north western Mediterranean sea. Deep Sea Res. II , 49/11, 1987-2005

[1] E-phytol was purified (Sims and Pettus, 1976) from purchased phytol (Aldrich) and phytyldiol was synthesized as described by Rontani *et al.* (1994).