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2-Ethyl-3-methylmaleimide in Tokyo Bay Sediments Providing the First Evidence for its Formation from Chlorophylls in the Present Photic and Oxygenic Zone

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Tokyo Bay bottom sediments were analyzed for 2-ethyl-3-methylmaleimide, a degradation product of chlorophylls, which has been detected in ancient sediments. It was found in all sediments examined in concentrations of about 1 to 15 nmol/g- of dried sediment, and it was shown to be preserved for 100 years in the sediments. Its depth distribution agreed with that of the reported total organic carbon content of the sediments, reflecting a change in primary productivity. We concluded that this maleimide was produced under photic and oxygenic conditions in nature before the incorporation of photosynthesizing organisms into sediments.

Key words: maleimide; porphyrin; chlorophyll; sediment

Portions of biomolecules that have ceased to play their roles in living organisms are deposited in sediments. Some of these molecules are decomposed to simple molecules, mainly by the action of microorganisms in the sediments, but others can remain in sediments for geological periods, undergoing minor or major modification by chemical transformation. Consequently, these molecules become one of the objects in the field of organic geochemistry. It is important to understand the transformation processes of biomolecules in sediments, to provide information on the histories of the molecules and their sedimentary environments. For instance, from knowledge of molecular transformations, such as racemization of L-amino acids to D and decarboxylation of *n*-fatty acids to *n*-alkanes in sediments, evolved the concepts of the molecular clock and the carbon preference index, respectively.¹⁻³⁾ The preserved biomolecules that have undergone only limited modification and can be unambiguously linked with precursor compounds or further with specific organisms are called biomarkers; examples are porphyrins in crude oils and an-

Since the discovery of porphyrins in sediments by Treibs in 1934,⁴⁾ these compounds have been widely studied as biomarkers for photosynthetic organisms. 2-Ethyl-3-methylmaleimide (1) was recently found in Permian and Triassic marine sediments by Grice et al.,⁵⁻⁶⁾ and in Neogene and Cretaceous/Tertiary sediments by us.⁷⁻⁸⁾ together with other alkylmaleimides. The compound is a novel biomarker for chlorophylls, because many kinds of chlorophylls contain a 3-ethyl-4-methylpyrrole moiety. No other precursors in nature have been known to afford the 2-ethyl-3-methylmaleimide structure, as far as we know. Higher plants and phytoplankton are the major contributors to terrestrial and marine sedimentary organic matter, respectively, and the sedimentary maleimide, therefore, is presumably derived from chlorophylls of photosynthetic organisms. An advantage of the use of this new biomarker compound is that it can be extracted more easily from sediments and identified by GC-MS than porphyrins can be.

The transformation of maleimides from pyrrole moieties of chlorophylls occurs under photic or oxygenic conditions or both. Therefore, the occurrence of the maleimide in ancient sediments raised



cient sediments derived from chlorophylls or photosynthetic organisms.

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the issue of the conditions and timing of the oxidation of tetrapyrrole pigments, because ancient sediments do not provide such oxidative conditions. In this study, on the assumption that the maleimide is formed in photic or oxygenic environments and preserved in the sediments, we undertook a search for maleimides in the sediments of Tokyo Bay, the depositional environment of which is apparently oxidative.

Materials and Methods

General procedure for analysis of maleimide in Tokyo Bay sediments. The Tokyo Bay bottom sediments that we analyzed were portions of a 100-cm core collected at about 5 km offing from the influx of the Tama River in 1986. The core was cut every 2.5 cm from the surface to 30 cm in depth and every 5.0 cm from 30 to 100 cm in depth. These layers were lyophilized, pulverized, and kept in a freezer until analysis. The samples (0.4 g) were extracted by sonication with *n*-hexane, a 7:3 mixture of *n*-hexane and CH₂Cl₂, CH₂Cl₂, a 4:1 mixture of CH₂Cl₂-methanol and methanol (2 ml each) 6 times each, and each extract was put of a silica gel column $(175 \times 10 \text{ mm i.d.})$ with *n*-hexane, CH_2Cl_2 , CH_2Cl_2 -acetone (9:1), and methanol (12 ml each) as eluents. The eluent was concentrated to 50 μ l under a stream of nitrogen. One microliter of a 50- μ l solution was analyzed by GC-MS.

For analysis of dihydrohematinic acid (3) or hematinic acid (4), the fraction obtained from 1 g of surface sediment was concentrated to dryness and treated with 15% BF₃-methanol (1 ml) at 80°C for 20 min. The reaction mixture was then concentrated to dryness, dissolved in CH_2Cl_2 , and analyzed by GC-MS.

The samples were analyzed with a Hewlett Packard G1800A GCD system. The GC was equipped with an FFAP bonded FS-WCOT capillary column (60 m × 0.25 mm i.d.). The oven temperature was programmed to give 50°C for 2 min, to increase from 50°C to 150°C at the rate of 10°C min⁻¹, then from 150°C to 230°C at the rate of 3°C min⁻¹, and to hold at the final temperature for 50 min. Helium was used as a carrier gas with a flow rate of 1 ml min⁻¹. Electron impact (70 eV) mass spectra were obtained by scanning from m/z 45 to 425 every 0.5 s.

Identification of 2-ethyl-3-methylmaleimide was made by comparison of the retention time on the GC and of the MS fragment patterns with those of the standard compound, which was synthesized through a new easier path as stated below. The abundance of maleimide was estimated by comparison of the peak area on a mass fragmentogram of molecular ion to that of the standard compound.

Preparation of authentic 2-ethyl-3-methyl-



Fig. 1. Total Ion Chromatogram (a) and Mass Fragmentogram (b) of an Extract of Tokyo Bay Sediment and Total Ion Chromatogram of Authentic 2-Ethyl-3-methylmaleimide (c).

maleimide (1). Diethyl 2-ethyl-3-methylmaleate was prepared by a similar method to that reported for the stereoselective synthesis of diethyl 2-butyl-3methylmaleate,⁹⁾ from diethyl 1-ethoxycarbonylpropane-1-phosphonate and ethyl pyruvate as a colorless oil in the yield of 56%. ¹H-NMR (CDCl₃) δ : 1.08 (3H, t, J=7.5 Hz, 2-CH₂CH₃), 1.30, 1.32 (total 6H, t×2, J=6.5 Hz, OCH₂CH₃), 1.96 (3H, s, 3-CH₃), 2.38 (2H, q, J=7.5 Hz, 2-CH₂CH₃), 4.20, 4.22 (total 4H, q×2, J=6.5 Hz, OCH₂CH₃).

The maleate thus obtained (2.1 g, 10 mmol) in ethanol (5 ml) was treated with 2 M NaOH (5 ml) at 40 °C for 3 h. The solution was acidified with 2 M HCl at 0 °C and extracted with ether. The extract was concentrated and the residual oil was reacted with urea (2.0 g) at 100 °C for 1 h. The resulting solid was extracted with chloroform and the extract was purified by silica gel column chromatography with benzeneethyl acetate as the eluent to give the desired maleimide (1) as needles (0.59 g, 43%), mp 67-8°C (lit. 68°C). ¹H-NMR (CDCl₃) δ : 1.15 (3H, t, *J*= 7.6 Hz, 2-CH₂CH₃), 1.98 (3H, s, 3-CH₃), 2.42 (2H, q, *J*=7.6 Hz, 2-CH₂CH₃), 8.30 (1H, broad s, NH). *Anal*. Found: C, 60.46; H, 6.55; N, 9.97%. Calcd. for C₇H₉O₂N: C, 60.42; H, 6.52; N, 10.07%.

Results and Discussion

Each of 16 samples of the divided core was extracted successively by sonication with *n*-hexane, *n*hexane-CH₂Cl₂ (7:3), CH₂Cl₂, CH₂Cl₂-methanol (4:1), and methanol. The extracts were each separated by silica gel column chromatography, each fraction being analyzed by GC-MS.

2-Ethyl-3-methylmaleimide (1) was detected in the CH_2Cl_2 -acetone fractions of the CH_2Cl_2 -methanol extracts of all the samples analyzed. Typical GC-MS data of the extract are shown in Figs. 1 and 2, together with those of an authentic sample, which



Fig. 2. Mass Spectra of Maleimide in the Extract of Tokyo Bay Sediment (a) and Authentic 2-Ethyl-3-methylmaleimide (b).

certified the identification of 1. A search for other expected oxidation products of chlorophylls, 2-methyl-3-vinylmaleimide (2) and dihydrohematinic acid (3), or hematinic acid (4), was also done by GC-MS, and among them, only 4 was found as traces in the methanol fraction of the CH_2Cl_2 -methanol extract after esterification. The source of 4 seems ambiguous, as it can be formed both by oxidation of chlorophylls accompanying dehydrogenation and by oxidation of heme.

Jen and Mackinney reported the formation of 2ethyl-3-methylmaleimide (1) by sunlight irradiation of chlorophyll a in organic solvents, the photodecomposition accompanying oxygen uptake of 3 moles or more per chlorophyll molecule.¹⁰⁾ They found no other maleimides or related compounds, such as 2, 3, or 4, in the reaction mixture. On the other hand, the enzymatic breakdown pathway of chlorophylls has recently become clearer, and an oxygenolytic opening of porphyrin rings was found to occur in senescent leaves between C-4 and C-5 of chlorophylls (see Scheme 1).¹¹⁻¹²⁾ However, no reports have appeared about the enzymatic production of maleimides from chlorophylls. Therefore, oxygen-assisted photodecomposition of chlorophylls or their enzymatic catabolites might contribute to the formation of the 2-ethyl-3-methylmaleimide (1) that we detected in Tokyo Bay sediments. This means that maleimide generation probably occurs in dead organisms before they are buried in the deposits of the Tokyo Bay bottom. The practical absence of 2, 3, or 4 in the sediments is consistent with the previous report.

To examine the preservation of 1 in the sediments, we analyzed the depth distribution of the quantity of 1 throughout the core, as shown in Fig. 3, together with the depth profile of the total organic carbon content (TOC) of the sediments.¹³⁾ Included in Fig. 3 for comparison is the depth distribution of the total amount of chlorophyll a and related compounds (pheophytin a, pyropheophorbide a, and pyropheophytin a); the details of analysis for these com-



Fig. 3. Depth Distributions of 2-Ethyl-3-methylmaleimide and Total Amount of Chlorophyll a and Related Compounds (a) and Total Organic Carbon Contents¹³⁾ (b) in the Tokyo Bay Sediments.

pounds will be reported elsewhere. The linear sedimentation rate had been assumed to be 1.5 cm/year from the surface to 25 cm in depth and 1.0 cm/year below 25 cm in depth.¹³

The sediments below 30 cm in depth were found to contain maleimide 1 in almost equal concentration (about 2 nmol/g), and a rather steep increase of the maleimide content (from 2 to 13 nmol/g) was observed from the 20-cm- to the 30-cm-depth sample. The reported TOC increase from the bottom to the 30-cm-depth sample has been attributed to the increase in organic pollutants corresponding to population growth in the metropolitan area. The higher and almost uniform TOCs of the upper sediments had been considered to reflect both the pollutant influx and the increased propagation of phytoplankton caused by advanced eutrophication, the latter having operated about 10 years later than the former. The increase in the maleimide content being delayed behind that of TOC suggests that maleimide 1 found in the sediments originates from phytoplankton rather than from terrestrial plants.

This analytical study provides the first evidence for formation of 2-ethyl-3-methylmaleimide from chlorophylls under photic and oxygenic conditions in nature. This maleimide was found to be preserved for about 100 years in the Tokyo Bay sediments, and its depth distribution seemed to reflect a change in primary productivity. In our previous study, Cretaceous/Tertiary sediments proved to contain no 2-methylmaleimide in spite of the existence of methylpyrrole-containing porphyrins in appreciable amounts.⁸⁾ This fact may rule out the possibility of

1846

diagenetic generation of maleimides from porphyrins in sediments. The maleimide detected in the ancient sediments could have been produced before the incorporation of dead bodies of photosynthesizing organisms into sediments, rather than from sedimentary porphyrins by the action of diagenesis in sediments.

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