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Biological formation of ethane and propane in the deep marine subsurface

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Contributed by John M. Hayes, July 31, 2006

Concentrations and isotopic compositions of ethane and propane in cold, deeply buried sediments from the southeastern Pacific are best explained by microbial production of these gases *in situ*. Reduction of acetate to ethane provides one feasible mechanism. Propane is enriched in ¹³C relative to ethane. The amount is consistent with derivation of the third C from inorganic carbon dissolved in sedimentary pore waters. At typical sedimentary conditions, the reactions yield free energy sufficient for growth. Relationships with competing processes are governed mainly by the abundance of H₂. Production of C₂ and C₃ hydrocarbons in this way provides a sink for acetate and hydrogen but upsets the general belief that hydrocarbons larger than methane derive only from thermal degradation of fossil organic material.

ethanogenesis | hydrocarbon gases | marine sediments |
propanogenesis | stable carbon isotopes

Leg 201 of the Ocean Drilling Program was dedicated to the study of microbial life in deeply buried marine sediments (1, 2). Cores were obtained from open-ocean sites in the Equatorial Pacific, where sediments deposited 40 million years ago are underlain by seafloor basalts through which oxygenated seawater is flowing, and from the Peruvian Margin, where drilling penetrated sediments up to 15 million years old (Fig. 1). Temperatures in sediments ranged from 2°C to 25°C. All sites are isolated from reservoirs of fossil hydrocarbons. At both open-ocean and ocean-margin sites, treatment of sediments with strong base released ethane and propane (Fig. 2). When the treatment was repeated with fresh sediment and isotopically labeled water ($\delta D = +4000\text{‰}$), no excess deuterium appeared in the ethane or propane. Therefore, we conclude that the hydrocarbons were strongly sorbed, indigenous constituents of the sediment and did not derive from a chemical reaction between the strong base and an organic substrate.

Earlier reports describe sediments offshore Peru (3) and Spitsbergen (4), from which similar mixtures of hydrocarbons could be released by treatment with hot solutions of phosphoric acid. In each case, the carbon-isotopic compositions and abundance ratios (C₁/C₂₊) led to reluctant suggestions that the gases must be of thermogenic origin and thus have migrated into the unconsolidated seafloor sediments: “the [postulated] migration of C₂₊ hydrocarbons... is somehow related to these fluids [brines that might have flowed from one basin to another]” (3); and “. . . elevated seepages [of thermogenic hydrocarbons] occurred irregularly but are not currently active... it remains speculative whether the detected hydrocarbon anomalies are related to reservoirs and/or active source rocks” (4). No mechanism for sorbing the putatively migrated hydrocarbons more strongly than indigenous microbial products has been offered.

Ethane and propane with similar isotopic characteristics and abundance ratios have recently been reported in Cretaceous marine shales in the Western Canadian sedimentary basin (5). Previous work has also pointed to the existence of microbially

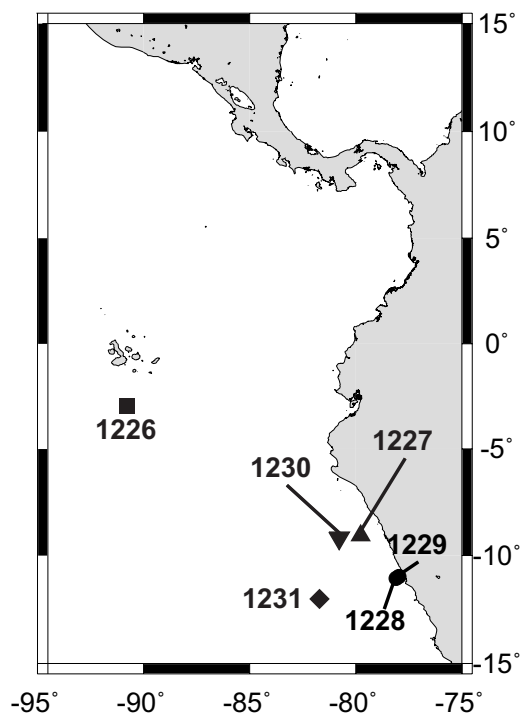


Fig. 1. Map showing the locations of studied ODP drill sites.

mediated pathways that yield hydrocarbons with two or more carbon atoms (6–12). In the present case, the occurrences of ethane and propane in each core are well correlated with pertinent biogeochemical factors and chances that migration could supply the ethane and propane are profoundly more remote. Accordingly, we explore the possibility that the gases are previously unrecognized products of the subsurface microbial community.

Results and Discussion

Distribution of Ethane and Propane in Sediments. The gases were detected in all depth zones studied, to 380 m below sea floor (mbsf; Fig. 2). Within each borehole, concentration profiles are more consistent with production *in situ* than with transport from

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The authors declare no conflict of interest.

Abbreviation: mbsf, m below sea floor.

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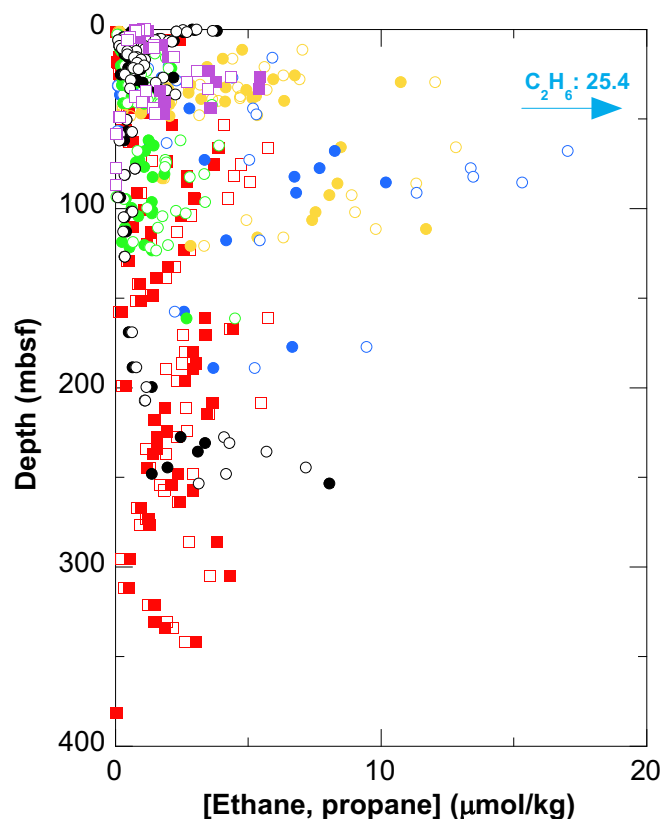


Fig. 2. Concentrations of ethane (filled symbols) and propane (open symbols) in sediments at ODP Sites 1226–1231, Equatorial Pacific and Peru Margin, expressed in μmol per kg of dry sediment: Site 1226 (red squares), Site 1227 (orange circles), Site 1228 (blue circles), Site 1229 (green circles), Site 1230 (black circles), and Site 1231 (purple squares). Sediments were deposited under a wide range of environmental conditions. In most samples, the bulk of the ethane and propane was sorbed to the sediment, i.e., dissolved ethane and propane were below the detection limit.

greater depths. Intact prokaryotic cells are present in all sediments studied and chemical compositions of pore fluids indicate microbial activity at all depths (1, 2, 13). DNA- and RNA-based culture-independent studies of the compositions of microbial communities in these sediments indicate the presence of diverse Archaea and Bacteria with largely unknown properties. Many of these phylotypes are apparently widespread in subsurface environments (14–17). The geochemical environments probed are highly diverse. Concentrations of organic carbon range from $\approx 0.1\%$ at the open-ocean Sites 1226 and 1231 to 12% at near-shore Sites 1227–1230, underlying highly productive surface waters off Peru (18). Concentrations of dissolved methane vary between sites by at least six orders of magnitude (1). Sulfate is consumed by respiration at relatively shallow depths at sites off Peru but is supplied to deeper sediments at Sites 1228 and 1229 by subsurface brines. It is never fully consumed at open-ocean Sites 1226 and 1231. Despite this high geochemical diversity, concentrations of ethane and propane are rather uniform and range from ≈ 1 to $25 \mu\text{mol}$ per kg of dry sediment (Fig. 2). Average concentrations of ethane and propane are highest at coastal Sites 1227, 1228, and 1229 (Table 1, which is published as supporting information on the PNAS web site), intermediate at the open-ocean Sites 1226 and 1231, and lowest at the organic-carbon-rich, methane-hydrate-bearing Site 1230. At Sites 1226 and 1228, ethane and propane together account for roughly as much carbon as methane (Table 1). They account for 30-fold less carbon than methane at Site 1231 and still less at Sites

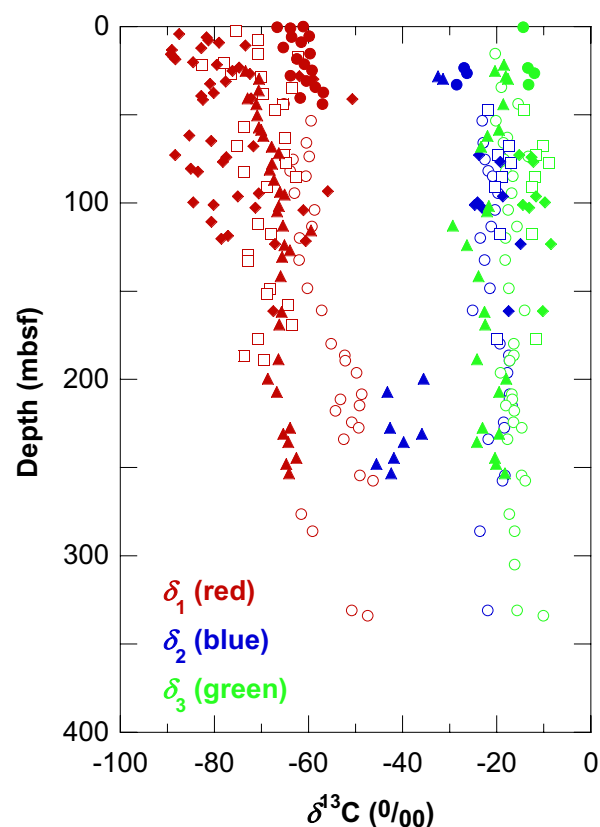


Fig. 3. Carbon-isotopic compositions of methane (δ_1 , red symbols), ethane (δ_2 , blue), and propane (δ_3 , green) at ODP Sites 1226 (open circles), 1228 (open squares), 1229 (diamonds), 1230 (triangles), and 1231 (filled circles) [$\delta_n = (^{13}R_n/^{13}R_{\text{vpdb}}) - 1$, where $^{13}R \equiv ^{13}\text{C}/^{12}\text{C}$ and vpdb designates the Vienna Pee Dee Belemnite isotopic standard. Reported values of δ are customarily multiplied by 1,000 and expressed in permil units (‰).] Note that δ_1 reflects the isotopic compositions of mixtures of sorbed and dissolved methane with variable relative proportions (e.g., Site 1226, $\text{CH}_{4,\text{aq.}} < \text{CH}_{4,\text{sol.}}$; Site 1228: $\text{CH}_{4,\text{aq.}} < \text{CH}_{4,\text{sol.}}$; Site 1229: $\text{CH}_{4,\text{aq.}} \approx \text{CH}_{4,\text{sol.}}$; Site 1230: $\text{CH}_{4,\text{aq.}} > \text{CH}_{4,\text{sol.}}$; Site 1231: $\text{CH}_{4,\text{aq.}} \ll \text{CH}_{4,\text{sol.}}$), whereas δ_2 and δ_3 largely pertain to the sorbed fraction of the respective gases.

1227, 1229, and 1230. At all sites except 1230, the combined concentration of sorbed ethane and propane is comparable to or higher than the sum of dissolved, volatile fatty acids (1).

Sites 1226 and 1231 are remote not only from continental sources of hydrocarbons but also from the active margin and thick piles of sediments (Fig. 1). At these sites, relatively thin packages of cold, organic-lean sediment overly a basement through which oxygenated seawater circulates. The ethane and propane must have been produced *in situ*, and microbial catalysis is, by far, the most likely source. At Site 1231, where ethane and propane were detected in the top 47 mbsf (Fig. 2), the concurrent presence of dissolved manganese, dissolved iron (1), and sorbed methane with $\delta^{13}\text{C}$ of -57 to -65‰ (Fig. 3), testifies to microbial activity in this sediment interval.

Similarly, at Site 1230, discrete maxima of both hydrocarbons were observed at specific sediment horizons and appear to be linked to microbial processes. Here, concentrations of ethane and propane are bimodally distributed, with peaks of $\approx 4 \mu\text{mol}/\text{kg}$ at the sediment surface and $\approx 8 \mu\text{mol}/\text{kg}$ at ≈ 250 mbsf. In the deep sediment interval, a relatively high proportion of the gases was dissolved in porewater rather than sorbed to the sediment because it was already detected by applying extraction protocols that are largely limited to dissolved gases (1). The surface maximum is best explained by a coupling of hydrocarbon

Assuming that the isotope effects associated with reduction, ε_2 and ε_3 , are larger than ε_5 and ε_6 , respectively, increasing values of x are associated with increasing values of δ_{DIC} and/or f_3 . In fact, higher values of x (see reference lines, Fig. 5B) are associated with samples that have higher values of δ_{DIC} (Table 2). Conversely, lower values of x tend to be associated with samples containing higher concentrations of sulfate (Table 2). The latter should favor oxidation rather than reduction of C_3^* and thus minimization of f_3 . Notably, these relationships prevail over a wide range of concentrations (3–160 mM) and isotopic compositions (-20 to $+20\%$) of DIC (D. P. Schrag, unpublished data, compare Table 2).

Values of δ_2 decrease linearly with $\log[\text{acetate}]$ (Fig. 5C). Strikingly, the relationship pertains to concentrations of acetate varying by a factor of 10,000. It would be relatively easy to explain if the system were closed and acetate could be viewed as a reactant that was being consumed by some process with a normal isotope effect. In such cases, the δ value of the unconsumed acetate would increase linearly with declining values of $\log[\text{acetate}]$. In this scenario: (i) Acetate, or some precursor molecule from which acetate is derived, is consumed by a process with a normal isotope effect. Its isotopic composition therefore follows the relationship shown in Fig. 5C. (ii) When that process stops, a portion of the residual acetate is converted to ethane. The isotopic composition of the ethane therefore monitors that of the residual acetate. This hypothetical sequence deserves attention because it is relatively simple and fits the observations so perfectly. Only further study can determine whether it should be discarded, modified, or accepted.

In an open system, the relationship summarized in Fig. 5C might indicate mixing of ethane from two sources, with the mixing ratio somehow related to $\log[\text{acetate}]$. There are, for example, two processes, fermentation and homoacetogenesis, which produce isotopically distinct acetate (31, 32). The resulting variations in δ_{acetate} would, after reduction of the acetate to produce ethane, appear as variations in δ_2 . The processes might be complementary because fermentation produces H_2 and acetogenesis consumes it. A logarithmic dependence on $[\text{H}_2]$ would be expected because relative rates of metabolic processes in marine sediments often depend on their respective free energy yields (33). To obtain the relationship in Fig. 5C it is then required that the concentration of acetate monitors that of H_2 . Again, further study is required.

Sorbition of Ethane and Propane. The combination of low concentrations of ethane and propane in interstitial water and the requirement to use strong base to release them suggests that these hydrocarbons, including methane and sometimes also their larger homologues, were adsorbed to hydrophobic siloxane patches within the interlayer region of clay minerals (34). Expandable clay minerals such as smectite are common constituents of marine sediments and are considered important host phases of organic carbon (35) and potential nucleation sites for gas hydrates (36).

Conclusion

Previous studies have provided independent lines of evidence in support of biologically mediated pathways leading to gaseous hydrocarbons other than methane (6–12). Previous work had focused mostly on ethane and demonstrated that (i) certain methanogenic archaea (7) may be capable of ethane production, (ii) ethylated Coenzyme M (ethyl-S-CoM) can play a mechanistic role (7), and (iii) ethanethiol can serve as a substrate (9). However, propanethiol could not be confirmed as substrate for propane production (9), and mechanisms for its biological production are not known. In connection with the evidence presented here, this finding implies that multiple

substrates and mechanisms are associated with the formation of hydrocarbons.

Although concentrations of biogenic ethane and propane are low, the likely significance of these compounds is great. Specifically, they signal the presence of an additional process, probably significant in many environments, for extending the terminal degradation of organic material.

Materials and Methods

All sediments studied were recovered during Leg 201 of the Ocean Drilling Program (1). Details of the sampling and analysis of hydrocarbon gases are reported elsewhere (37) and in *Supporting Text*.

Concentrations of Hydrocarbon Gases. In brief, hydrocarbon gases were analyzed by using a headspace technique. Three-ml subcores of fresh sediment were taken immediately after core recovery on the catwalk and placed in headspace vials containing 5 ml of 1 M NaOH. Mixtures were shaken for 1 h; afterward the sediment and the NaOH solution had typically formed a slurry. Sampling of the headspace with a gas-tight syringe was performed at least twice within 4 months after core retrieval. Analysis of the hydrocarbons was performed with a Hewlett Packard 6890 gas chromatograph equipped with a stainless steel column packed with HayeSep S (100–120 mesh) and equipped with a flame ionization detector (FID). Gas chromatography onshore used a Hewlett Packard 5890 gas chromatograph equipped with Poropak-Q column. A calibration resulting from injection of known quantities of hydrocarbon gases was applied to calculate the molar fraction of ethane and propane in the gas mixture. Further details can be found in *Supporting Text*.

Isotopic Compositions. Headspace gas samples were sampled by using a 1,000- μl gastight syringe and injected into a six-port 2-position valve with a 500- μl external loop upstream of a Gerstel CIS-4 injector on an HP6890 gas chromatograph that was operated in split mode. The split ratio was adjusted to optimize the signal intensity, typically between 0.1 and 1.0. Gaseous hydrocarbons were separated on a 30 m \times 0.3 mm ID Alltech AT-Q column with helium carrier gas flow of 3.0 ml/min. The column temperature was programmed from 50°C with a 2-min hold at 50°C per min to 240°C. An integral fused silica combustion system (38) at 950°C converted all organic components to CO_2 . Isotopic data were acquired and processed on a Finnigan MAT Delta Plus isotope-ratio-monitoring mass spectrometer using the Isodat NT data package. Overall system accuracy was confirmed to be better than 1‰ based on a standard with ethane and propane in helium at 1,000 ppm (nominal). The standards had been independently analyzed by Isotech (Champaign, IL).

Thermodynamic Calculations. The standard free energies (ΔG°) of acetotrophic ethanogenesis and propanogenesis were calculated by using SUPCRT92 (39) and thermodynamic data from Shock and Helgeson (40) for a pressure of 20 MPa and temperatures as measured in the boreholes (1). The free energy of reactions at nonstandard conditions (ΔG) was calculated according to $\Delta G = \Delta G^\circ + RT \ln Q$, where Q is the activity quotient of the reactants and reaction products. For reaction 2, for example, $Q = [a_{\text{propane(aq)}} \times a_{\text{H}_2\text{O}}^5] / [a_{\text{acetate(aq)}} \times a_{\text{HCO}_3^-} \times a_{\text{H}_2\text{(aq)}}^6 \times a_{\text{H}^+}^2]$. R is the gas constant, and T is the temperature in Kelvin.

For the construction of Fig. 4, the dependency of aqueous acetate activity on H_2 activity was calculated for constant ΔG^{R} values of 0 and -15 kJ/mol, assuming $\text{pH} = 8$, $a_{\text{HCO}_3^-} = 10$ mM, $a_{\text{ethane}} = a_{\text{propane}} = 20$ nM, and $T = 20^\circ\text{C}$ and using

