Biomarker Identification in a Latex Fossil

Leo W. Collins
O.I. Analytical Corporation

INTRODUCTION

Partially coalified, structurally intact latex fibers found in a low rank coal were characterized by analytical methods. The latex fibers have a high sulfur content of approximately 10 percent. It has been proposed in previous studies, and it appears to be the case here, that the latex material has been “naturally” vulcanized during the early diagenetic period. Two fossilized latex samples recovered from different locations within the Geisaltal brown coal deposit in Germany were characterized, and the same major compounds were found in both samples with slightly varying concentrations. Pyridine extracts of the samples were analyzed by capillary gas chromatography (CGC), combined capillary gas chromatography-mass spectrometry (GC-MS), and low voltage high resolution mass spectrometry (LVHRMS). Twelve amyrin and hopanoid bio-marker derivatives were tentatively identified in the extract by comparing the Kovats retention indices, experimental mass spectra, and order of elution with those in the literature. Kovats retention indices for the 12 compounds are reported. Three identifications were verified by co-chromatography with authentic standards. The amyrin derivatives are indicative of all rubber plants, while the appearance of hopanoid derivatives lends support to the role of microorganisms in diagenesis.

Compounds of biological origin, or biomarkers, are ubiquitously found in fossil fuels and sediments. These compounds can facilitate the understanding of the diagenetic process and provide clues in petroleum exploration and geochemical

Brunauer, Emmett and Teller — The Personalities Behind the BET Method

Burtron Davis,
Center for Applied Energy Research

The first portion of Dr. Davis’ article appeared in the last issue of Energeia

PART II

Paul Emmett (1900 - 1985)

Paul Emmett arrived in Washington in 1926 to work at the Fixed Nitrogen Laboratory (FNL) because he missed the research days of graduate school. Emmett was born in Portland, Oregon. His father, a railroad worker, was frequently absent from home and Emmett was raised primarily by his mother. When he went to Cal Tech to obtain his Ph.D., his mother accompanied him to Pasadena to establish a home there. Nobel Prize winner Linus Pauling was a fellow student with Emmett in both high school and undergraduate days, and lived with the Emmetts for a year.

Cal Tech was a small school just starting graduate studies; only four Ph.D.s had been awarded prior to the class of four that included Linus Pauling and Paul Emmett. These were not two ordinary graduate students. They published a paper in the prestigious Journal of the American Chemical Society while graduate students, and without including a faculty member. Emmett returned to Portland to teach at Oregon State University, but missed research work. He arrived at the FNL just as the direction of the lab was turning from development work on the synthetic ammonia catalyst to that of understanding the mechanism of the catalytic reaction. Emmett was a brilliant organizer and, despite his low-key personality, an intense competitor. He was an outstanding writer and a dynamic speaker who was an effective salesman of the BET method. In addition to the work at the FNL that led to the BET equation, Emmett led a team consisting of Brunauer and five other professionals, in defining the kinetics and mechanism of the ammonia synthesis. In science where change is the final objective, the understanding of ammonia synthesis developed by Emmett and his coworkers is just as valid today as it was when the work was carried out.

Teller (1908 - )

Edward Teller, like Brunauer, was born in Hungary. He immigrated to the U.S. at the inducement of George Gamow. The President of
Biomarker, (continued)

The compounds were extracted from a fossil latex sample found in the Eocene brown coal deposits of the Geiseltal formation near Halle, Germany. Compound identification was made by comparing the Kovats retention indices to literature values, comparing the mass spectral data to published spectra, and interpreting the mass spectra. Other researchers have used gas chromatography and gas chromatography-mass spectrometry methods to identify biomarkers in coal, petroleum, aerosols, and sediments.

The fossil latex samples were taken from thick mats of hairlike cellular remains in Eocene brown coal deposits. The preservation is attributed to the unusual geological conditions of the Geiseltal and to the chemical stability and resistance to degradation of rubber. Findings of chemically and morphologically similar fossils to those in the geological record are rare. The latex samples taken from this brown coal seam may not be representative of the entire seam.

The latex material has been analyzed for sulfur and contained an unusually high amount, close to 10 percent. The sulfur forms of this material have been determined by S L-edge X-ray absorption near edge spectroscopy (XANES), and X-ray photoelectron spectroscopy (XPS). The XANES results indicated the presence of 40-50 percent thiophenic sulfur, 20-40 percent per cent aryl sulfide, and 20-30 percent disulfide forms in the latex samples. XPS results suggest that the latex material contains 49-55 percent organosulfides, 20-43 percent thiophenic sulfur, 0-8 percent sulfate/sulfone, and 0-13 percent pyrite.

Two classes of terpenoid derivatives were identified in the extracts of the fossil laticifers. They are amyrin derivatives and a family of hopanoids. The amyrin derivatives are likely the immature coalified products of α or β amyrin, which are commonly found in plants that produce latex. Hopanoids have been found in almost every type of organic sediment, including fossil fuels.

EXPERIMENTAL

The fossil latex samples, Neumark Sud #5 and Neumark Sud #13, were obtained from the Geiseltal Museum of Martin Luther University in Halle, Germany. They were pulverized and sonicated for one hour at room temperature in reagent grade pyridine. The mixture was filtered through 0.45 µm cellulose filter and the volume reduced to 5 ml. Separation of the extractable components was performed by temperature-programmed gas chromatography, using a Hewlett Packard 5890 gas chromatograph equipped with a flame ionization detector. Co-chromatography experiments with authentic standards were performed using the same conditions mentioned above.

Kovats retention indices were determined isothermally at 250 °C, using the same chromatograph and column mentioned above. The sample was introduced in the split mode with a 23:1 split ratio. Kovats retention indices were determined by adding a mixture of n-alkane bracketing standards and calculating the retention indices using the Kovats equation. The samples were run six times each. The retention times were calculated and the peak averages are reported below.

Gas chromatography-mass spectrometry experiments were performed using a Hewlett Packard 5890 Series II gas chromatograph and a Hewlett Packard 5898A mass spectrometer. High-resolution mass spectra were acquired using a Kratos MS-50 high-resolution mass spectrometer interfaced with a DS-55 data system.

RESULTS AND DISCUSSION

The chromatographic profiles of the pyrrole extracts of the Affenhaar samples Neumark Sud #5 and Neumark #13 appear in Figure 1. The numbered chromatographic peaks correspond to the appropriately numbered index in Table I, and identification in Table II. Note that the chromatographic profiles of the pyridine extracts of both Affenhaar samples are similar, but differ in the relative amounts of the various components. The identifications in Table II are at best tentative and are based solely on comparison of the experimentally determined mass spectra with those in the literature.

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RESULTS AND DISCUSSION

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![Figure 1](image)

Table I. Kovats Retention Indices

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>I&lt;sub&gt;250&lt;/sub&gt;</th>
<th>Standard Deviation (n=6)</th>
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<tr>
<td>I</td>
<td>2951.62</td>
<td>.03</td>
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<tr>
<td>II</td>
<td>2970.25</td>
<td>.04</td>
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<td>III</td>
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<td>.03</td>
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<td>XII</td>
<td>3167.63</td>
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Table II. Mass Spectrometry Results

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<th>Peak No.</th>
<th>Formula</th>
<th>Precise mass (amu)</th>
<th>Deviation from Calculated Mass</th>
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<th>P+2&lt;sup&gt;b&lt;/sup&gt; (%)</th>
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<td>0.0030</td>
<td>30.4</td>
<td>30.4</td>
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</table>

<sup>b</sup>Determined by high-resolution mass spectrometry.

<sup>b</sup>Determined by GC-MS using a low-resolution quadrupole instrument.
or by interpretation of the mass spectral data. The molecular formulae of some tentatively identified compounds were confirmed using high-resolution mass spectrometry performed by admitting the entire sample into the spectrometer. The precise masses, experimentally determined by high-resolution mass spectrometry, that correspond to the molecular formulae of compounds tentatively identified by GC-MS are listed in Table II. In some cases, the molecular formulae of compounds tentatively identified in were further confirmed by careful measurement of the isotope peaks of the parent ions. In other cases, this was not successful because no parent ion was observed or reliable measurements could not be obtained due to low ion abundance.

The Kovats retention indices of the chromatographic peaks in both samples were determined isothermally at 250 °C. The average index values and the standard deviations are listed in Table I. The Kovats retention index for compound V, 3028.27, is in fair agreement with a published value of 3031, as was the mass spectrum. To our knowledge, the literature contains no other references to Kovats retention indices at 250 °C.

The structures tentatively identified in the extract appear in Figure 2. Three hopanoid derivatives were separated and tentatively identified by comparison of the experimentally determined mass spectra to spectra in the literature. They are compounds I, VI, and XII. Nine amyrin derivatives were also tentatively identified. Compound V compared favorably with a published spectrum. Compounds VIII, IX, and XI are nominal mass of 342 amu. The mass spectra and elution order of these isomers matched a literature reference. Five isomers having a nominal mass of 376 amu were separated. Tentative assignments were made by interpretation of the mass spectral results. They are compounds II, III, IV, VII, and X. Co-chromatography and gas chromatography-mass spectrometry experiments of authentic standards allowed for the positive identification of compounds I, II, and VI to be made.

The analysis of this structurally intact latex fossil found in a young brown coal reveals hopanoid derivatives which are extremely close in structure to the original cell material of the microorganism involved in the biochemical process of coalification. This evidence enforces the theory of microbial action during diagenesis. The samples may not have been so well preserved had it not been for the presence of sulfur, contributing to the vulcanization of the plant material. This is shown in the integrity of the amyrin derivatives, which are also close in structure to their precursors.

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BET, (continued)

George Washington University wanted to develop an internationally known physics department and, seeking advise on how to do this, was told, “Develop a department emphasizing theoretical physics; its much cheaper than experimental physics.” Gamow accepted the position only if he could bring Teller along as an assistant. Teller obtained his Ph.D. working with Professor Werner Heisenberg, famous for his uncertainty principle.

While a student, Teller was to take a train to the country for an outing of mountain climbing. Deep in thought, he failed to notice that he should have changed trains until the one he was riding was again in motion. He leapt from the moving train, lost his footing on landing and, falling under the train, had one foot severed above the ankle. However, he did not let the accident prevent him from physical activities, including mountain climbing. He was an excellent table tennis player, beating everyone in the school except Professor Heisenberg, his mentor. It is not certain whether this speaks to Heisenberg’s (continued, page 4)
skill at table tennis, or to Teller's astute political sense.

Upon arriving in the U.S., Teller was one of the few people who had a background in quantum physics, which was in an early stage of development at the time. Thus, many of the most well-known scientists in the Washington area made their way to Teller's home to discuss research topics, and to obtain help from Teller.

Afterwards

Even before the BET paper was published the three scientists were going separate ways. Brunauer, following the publication of the BET paper, found himself a well-known person within an organization that wanted to de-emphasize theoretical studies of fertilizers, and to focus on other research areas. Thus, Brunauer was faced with saving his job in a time when his organization wanted to eliminate it. In his effort, Brunauer obtained contracts from the military, and defense work began to become more important to him. With the outbreak of World War II it became apparent to Brunauer that he would not be able to continue much longer at the FNL. He tried to join the military's Naval research effort. In his first attempt, he was turned down. Undaunted, Brunauer called upon his friends as well as those of his wife, to write letters in support of his second application. This resulted in a flood of letters from senators, ambassadors, etc., and in 1942 he became a Lt., Junior Grade, in the Naval Reserve. He was appointed to head a high explosives research group in the Bureau of Ordnance which, at the time, included only three people. Eventually this group expanded to more than 100 people, and included both Naval officers and civilian employees. By the end of the war Brunauer had become a Lt. Commander, and had led a research effort that made considerable progress in improving the surface and underwater explosives used by the Navy. Brunauer drew many well known scientists into the war effort; these included Albert Einstein and John von Neumann, the father of the computer. Brunauer remained in the same position following the war, but as a civilian employee. Shortly after the end of WWII Brunauer went to Hungary to develop a roster of Hungarian scientists. He was instrumental in helping many scientists come to the U.S. By 1950 he had been appointed to the highest grade level for civilian employees.

In 1944, Brunauer's wife Esther joined the State Department and rose quickly, in spite of the State Department not having females in high positions at the time. Esther was appointed by President Truman to the rank of Minister to represent the U.S. at the preparatory commission for the formation of UNESCO.

In 1950, Senator Joseph McCarthy launched his famous charge that there were 205 rank and file Communists in the State Department, and he waved a piece of paper that he claimed contained their identities. The Brunauers were on such a list. McCarthy listed Esther as the first person from the State Department who should be investigated. McCarthy turned to J. B. Matthews, a former investigator for the House Un-American Activities Committee, and was provided material about the Brunauers. Matthews had been a successful organizer of communist front organizations in the 1930's. He claimed to have met the Brunauers during the 1930, and that they were Communists. For some time thereafter the Brunauers were the subject of front page headlines. Esther was called to testify at the hearings of the Tydings Committee, and even William S. Buckley concluded that all of the evidence McCarthy presented about the Brunauers was incorrect. The result was that Stephen resigned from the Navy (Figure 1). Esther continued her fight, but a year later was terminated. The loyalty committee voted 2-1 that, while Esther was completely loyal, she was married to Stephen, a potential security risk because he had belonged to the Young Workers League.

Brunauer joined the Portland Cement Association in Chicago and his work made him an internationally known expert in this area. In Chicago, he became active in the Baptist Church, and soon became a Deacon. From 1952 until her death in 1958, Esther and Stephen remained together and were both active in church activities.

Emmett left the FNL in 1937 to become chairman of the Chemical Engineering Department at Johns Hopkins University. Here he was charged with obtaining professional accreditation for the newly-formed, small department. This he accomplished in three years. During World War II Emmett became involved with defense research. Initially this involved the development and evaluation of adsorbents for gas masks. He was soon called to the Manhattan Project at Columbia University. Here he reported to Harold Urey, winner of a Nobel Prize for separating the heavy hydrogen isotope, deuterium. Emmett's work at Columbia University involved the development of fluorocarbons to serve as barrier material and pump fluids for use in separating uranium isotopes using the diffusion process.

Teller, together with Gamow, established a leading research group in the Washington area, and emphasized theoretical research. The explanation of nuclear reactions being responsible for the sun's source of energy was put on a firm theoretical basis by the group's work. It was at a meeting sponsored by this group that Niels Bohr made the first announcement in the U.S. of the splitting of the atom. Teller, as the U.S. entered WWII, joined the Manhattan effort, first at Columbia University, then at the University of Chicago, and finally at Los Alamos. Even before the war was over, Teller had turned to the development of the H-Bomb, based on nuclear fusion and not the fission process used to construct the bombs dropped on Japan during the war. Teller is credited with developing the science and pushing the political process that led to the H-Bomb. As such, he became the "model" of a right-wing fanatic, especially during the period of unrest of the 1960-70s.

After the Fixed Nitrogen Laboratory years, the three came together only twice. They were awarded honorary Doctor of Science degrees from Clarkson University in 1969 (front page photograph) and ten years later, the three, along with their wives, had a quiet dinner together in San Francisco to celebrate their paper.

The three individuals involved with developing the BET method do not fit the public's image of scientists. In the scientific area they rank at the top of their profession. They exhibit personalities that can be found in the more dynamic people of any profession. Politically they represent various views, ranging from very liberal to very conservative. However, it would be unfair to label any of the three by their political or personal beliefs since these changed as they grew in years and experience. They came together for a short while to contribute a significant advancement to science and lived the rest of their lives following various pursuits.
Making Allowances: SO₂ Emissions Trading

Don Challman, Center for Applied Energy Research
University of Kentucky

The Emissions Trading Program, created under Title IV of the 1990 Clean Air Act Amendments, represents a textbook application of economics to pollution control - the creation of a market in tradeable emission “allowances” for sulfur dioxide (SO₂) emitted from fossil fuel-fired power plants. Economists have long argued that such an approach will achieve the same level of environmental control as prescriptive government regulations but at lower cost. Conceptually, the idea is simple: exact some cost for pollution, and let the marketplace decide the optimum combination of measures for reducing emissions.

In practice, the Emissions Trading Program combines both market and regulatory approaches. Emission sources are given a “limited right” to pollute up to a regulatory imposed ceiling; the 1990 Clean Air Act set this ceiling at 8.9 million tons of SO₂ per year, after the year 2000 (10 million tons per year below the 1980 levels). To effect the cap, the EPA will annually allocate 8.9 million tradeable “allowances,” each of which permits the holder to emit one ton of SO₂. Allowances can be used in the year they are acquired, “banked” for future use, or sold. Ideally, firms that find cheaper ways to reduce emissions through pollution control equipment or fuel-switching will sell their allowances to firms with higher abatement costs. In turn, firms with prohibitive costs will purchase additional allowances to offset higher levels of SO₂ emissions. In this manner, overall costs will be minimized.

Examining, first, the cap, the fundamental question is whether 8.9 million tons per year by 2000 is too little, too late or too much, too soon. The answer is puzzling considering that the scientific community is divided over the link between the levels of emissions and potential secondary environmental impacts of “acid rain.” There is convincing evidence that: oxides of sulfur and nitrogen are the precursor emissions of primary interest; emissions concentrations are increasing and fossil fuel-fired power plants located in the Midwest and Eastern United States are large contributors; and pollutants, in combination with meteorological factors, can be transported, transformed and deposited as acidic materials in sensitive receptor regions. What remains to be proven, however, is the relationship between the levels of acidic deposition and damage to, for example, aquatic life, field crops and forests, and human-made materials and structures. To what level must we reduce emissions to enhance environmental quality? And where is the practical control limit beyond which the costs outweigh the benefits?

A more practical question is will trading effect emissions reductions where they are needed and at an acceptable cost? Although it remains to be seen where emissions reductions will take place, presumably Eastern and Midwestern U.S. utilities with relatively low production costs will find it more economical to reduce emissions. The ability to reduce costs relates to how well the market performs and how active trading is. Among the factors that could cause it to perform below its potential is the uncertainty associated with utility regulatory practices and future environmental requirements.

Regulated utilities, which face both economic and environmental regulatory constraints, are expected to represent a significant segment of the market. The effectiveness of allowance trading for reducing costs will depend largely on how allowance purchases are treated by public utility regulators. That is, the accounting, tax and cost-recovery “rules” applied to utility investments will strongly influence the relative costs of competing options, and the choice of allowances versus other technological options.

The straightforward choice is the option with the lowest marginal cost of abatement. However, the “cost-of-service” regulation of utilities does not necessarily lead utilities to make cost-minimizing decisions. If the cost of purchased allowances cannot be passed along to rate-payers, the utility has little incentive to acquire them and instead may choose more costly, long-lived capital assets that are allowed a return on investment (e.g., pollution control or new generating capacity). If revenues from excess allowance sales do not accrue to the utility, then similarly it has no incentive to sell allowances. The utility may instead choose not to reduce emissions (and thereby earn additional credits) or, alternatively, to “bank” excess allowances to meet future load growth requirements. In this situation, neither a selling utility nor a buying utility has much incentive to trade in the market.

Further, the direction of future environmental regulations is uncertain, either with respect to controlling the precursor emissions of acid rain or other air pollutants. Title IV of the Clean Air Act specifies that emission allowances are not a property right, and as such, a utility could see the value of its SO₂ allowances changed or diminished by future rulemaking. Second, many plants are subject to more stringent Title I requirements and schedules for meeting the National Ambient Air Quality Standards (NAAQS), including greater control of nitrogen oxides.

Third, the prospect of provisions to control carbon dioxide and hazardous air pollutants looms in the future. The difficulty is that the technologies for controlling air pollutants are highly interdependent. The solutions fashioned today to control SO₂ may not necessarily be the optimum solutions for controlling multiple types of pollutants.

These factors, with others such as increased competition in the utility industry, illustrate the extraordinarily complex technical and economic problems faced by utilities. At best, the “rules of the game” for buying and selling allowances have not been clearly articulated; at worst, the “game” could be an entirely different one with the emergence of new environmental requirements. Faced with such exceptional uncertainty, the choice of a typical risk-averse utility will be to avoid major changes in its allowance holdings. Consequently, this pioneering application of economics may result in little more than, at best, a rudimentary market for emission allowances.
Impact of Hazardous Air Pollutants on Mineral Producers and Coal-Burning Plants in the Ohio Valley

March 19-21, 1995
Hyatt Regency Hotel
Lexington, Kentucky

This conference is organized by the state geological surveys of the Ohio Valley and will focus on Title III of the Clean Air Act Amendments of 1990 and the potential impact on the coal industry and coal-burning plants of the Ohio Valley.

Featured speakers will be from Federal and State agencies and industry.

To receive a brochure which outlines the full program of the conference, contact Mary Lou Johnson, Conference Coordinator, at 606-257-2846.

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