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## N-ALKANES IN "THEM THAR HILLS"

### ABSTRACT

*Normal alkanes containing from 8 to 37 carbons were found in 300-million-year-old limestone taken from the Mississippian Lodgepole formation in the Bridger Range of southcentral Montana. Normal alkanes were also detected in younger limestones from the same range. The predominance of odd to even carbon numbers that is often observed in sediments was not found.*

**Key words:** normal alkanes, limestones, Mississippian Lodgepole formation, Bridger Range, chromatography, mass spectrometry.

### INTRODUCTION

Almost thirty years ago Dr. C. C. Bradley, Dean of the College of Letters and Science at Montana State, informed E. W. Anacker that some of the limestones from Montana's Bridger Range in southcentral Montana emitted an asphaltic odor when broken. Shortly thereafter Anacker decided that an attempt to identify some of the organic compounds present in Bridger limestones would be worthwhile. He drafted the first six coauthors—quite youthful chemistry undergraduates at the time—to climb mountains, ostensibly for rock samples, and to initiate the research. The relatively recent acquisition of mass spectrometers by the MSU Chemistry Department and the presence of a knowledgeable operator and spectroscopist, L. J. Sears, have permitted confirmation of the first results.

Limestone is the most abundant non-clastic sediment and consists of a solid rock made up of the shells and the skeletal materials of lime-secreting plants and animals (Emmons et al. 1939:272). Its chemical composition is highly variable but with calcium

carbonate the dominant component (Mason 1966:155). Gehman (1962) summarized analyses on some 346 limestones ranging in age from late Tertiary to Cambrian, i.e., from approximately 70 million to 500 million years old. The mean organic content was 0.24 weight percent and the mean hydrocarbon content 98 ppm. Hydrocarbons make up one class of biomarkers (biological markers and chemical fossils are equivalent terms), i.e., organic compounds which have carbon skeletons that can be related to precursor molecules from specific organisms (Eglinton and Calvin 1967, Philp 1986).

Life extends back more than a half billion years, the age of the oldest limestone in Gehman's summary (1962). H. J. Sanders (1967) cites the work of Calvin and coworkers which argued for the biological origin of isoprenoids in Minnesota's 2.7-billion-year-old Soudan shale (Eglinton and Calvin 1967) and the findings (Barghoorn and Schopf 1966, Schopf and Barghoorn 1967) of the remains of algalike and bacteriumlike organisms in 3.1-billion-year-old South African carbonaceous shale. The discovery of Myers and McCready

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(1966) that bacteria can penetrate intact rock casts a small shadow on the accuracy of the latter observation.

The organic matter in sedimentary rocks can be divided readily into two or more fractions (Jeffery 1970, Brownlow 1979). The small percentage extractable with organic solvents such as chloroform, benzene, and carbon disulfide is called *bitumen*. Another small fraction known as the *humic carbon content* can be extracted from the residue with aqueous sodium hydroxide. Most of the organic material in the original rock remains with the mineral fraction after these extractions and is referred to as *kerogen*.

In the work reported here, we concentrated our attention upon the n-alkanes in bitumens present in limestones found on Sacajawea, a twin peaked mountain in the Bridger Range of southwestern Montana. The limestone on the northernmost summit—highest point in the range at 9669 feet—is in the lower Mississippian Lodgepole formation and about 300 million years old (McMannis 1955). Although samples from various sites on Sacajawea were collected and examined, the results reported here refer only to those taken from the top of the northernmost peak (samples #1 and #8) and from the ridge between the two peaks (age decreasing continuously from sample #1 to sample #7).

## EXPERIMENTAL

To avoid lengthy and detailed descriptions of techniques—some of which were modified during the project—we limit the majority of our comments to procedures which produced the results reported. Research and attendant results not specifically cited here were to a large degree repetitive and supportive in nature.

Before analysis each sample was sandblasted to remove surface material, broken into small chunks (mostly 2 to 3 centimeters in diameter) with no visible cracks, and then immersed in acetic or hydrochloric acid for a short time to

remove contaminants that might have been present on surfaces defined by cracks present in the original sample and not affected by the sandblasting. Prior to extraction and after thorough rinsing with distilled water and drying, the rock chunks corresponding to a given sample were ground to a fine powder.

Solvents were purified by fractional distillation and used only after found to be chromatographically pure. Cellulose thimbles used in the extractions were cleaned by recycling hexane through them while in Soxhlets for 48 hours or more. Fresh hexane was then recycled through them for an additional 12 hours. The absence of hydrocarbons other than hexane (shown chromatographically) was taken as evidence that the Soxhlets and thimbles were clean enough to be used for extraction purposes.

Sample #1 used in the initial chromatography experiments was extracted with a 67% benzene, 33% ethanol mixture and the others were extracted with hexane. All extractions were allowed to continue for at least 72 hours. Solutions obtained were concentrated by removal of solvent, first by fractional distillation and then with the aid of a stream of nitrogen.

Preparative chromatography involving Adsorbosil-3 (Applied Science Laboratories) was employed to separate the hydrocarbons from other classes of compounds in sample #1 through #7 extracts. Between 15 and 20 plates were required to isolate the hydrocarbons from a single sample. Hydrocarbon mixtures were chromatographed on a Beckman GC-4 gas chromatograph using temperature programmed runs of 100°C to 300°C @ 10°C/min. Sample #1 was run with a 1% OV-1 column, and samples #2 through #7 were run on a 17 percent S.E.-30 column with a Chromosorb 100-150 mesh support. Compounds were identified through comparisons of chromatograms obtained with rock hydrocarbons and

with a standard paraffin series containing a  $C_{28}$  marker. The incorporation of the marker allows one to generate tables of relative retention times for each component. The use of such relative retention times provides a precise and reproducible measurement of chromatographic components. It compensates for inter-sample variations in elution time arising from small changes in chromatographic conditions.

Sample #8 used in the mass spectrometry study (equivalent to sample #1 but collected some 25 years later) was extracted with hexane. The concentrate obtained by allowing solvent evaporation to occur at room temperature from the extract was examined without further treatment. The gas chromatography/mass spectrometry (GC/MS) experiments were conducted with a VG 70E-HF magnetic mass spectrometer and a Varian 3700 gas chromatograph equipped with a 60 meter DB-1 capillary column (J&W Scientific) having an inside diameter of 0.25 mm and a film thickness of 0.25  $\mu$ . The GC was temperature programmed from 60°C to 310°C at 5°C per minute. The mass spectrometer was scanned over the mass range of 35-550 mass units at a rate of 0.7 seconds per mass decade. Mass spectra, obtained by electron ionization (EI) using 70 eV electrons, were stored and processed by a VG 11-250 data system based on the DEC-PDP 11/73 computer. Spectral assignments were made by a combination of manual interpretation utilizing known EI fragmentation mechanisms and comparison to library reference data. There are a number of concise and informative descriptions of techniques and instrumentation used to study chemical fossils in the literature. Ounsson, Albrecht and Rohmer (1984) provided one of them.

## RESULTS AND DISCUSSION

Diagnostic thin-layer chromatography provided evidence for the presence of hydrocarbons and several other classes of compounds in the

limestone samples. Only the hydrocarbons received significant attention.

Figures 1 through 7 are chromatograms of the hydrocarbons from samples #1 through #7, respectively, separated from extracts by preparative chromatography.

The peaks in Figure 1 indicate the presence of normal alkanes ranging from  $C_{13}$  to  $C_{37}$  in limestone sample #1. Another chromatogram obtained from a temperature program of 60°C-300°C @ 10°C/min. on a 4-foot, 3.8 percent S.E. column with a F&M Biomedical gas chromatograph extends the range down to  $C_8$ . The chromatogram is similar to those produced from very old shales (350-million-year-old Antrim and 2.7-billion-year-old Soudan) in that there is no predominance of molecules with an odd number of carbon atoms (Eglinton and Calvin 1967). The predominance of normal alkanes with an odd number of carbon atoms such as found in the relatively young, 50-million-year-old Green River shale is a strong argument for the biological origin of the hydrocarbons (Eglinton and Calvin 1967). The lack of this predominance, however, is not a strong argument for a nonbiological origin. Koons *et al* (1965) found that normal alkanes from marine sources do not show any special odd to even preferences. Johns *et al* (1966) concluded in another study that an odd over even preference in ancient oils is unsatisfactory as a mark of biogenicity. The lack of predominance of the number of odd over even carbon atoms could be due in part to the slow cracking by heat of carbon chains over time. If Green River shale is heated above 300°C for many hours, the straight-chain alkanes show a reduced dominance of odd-carbon molecules (Eglinton and Calvin 1967). According to Philp (1986), the ratio of odd-numbered alkanes to even-numbered alkanes trends toward a value of 1 with increasing maturity.

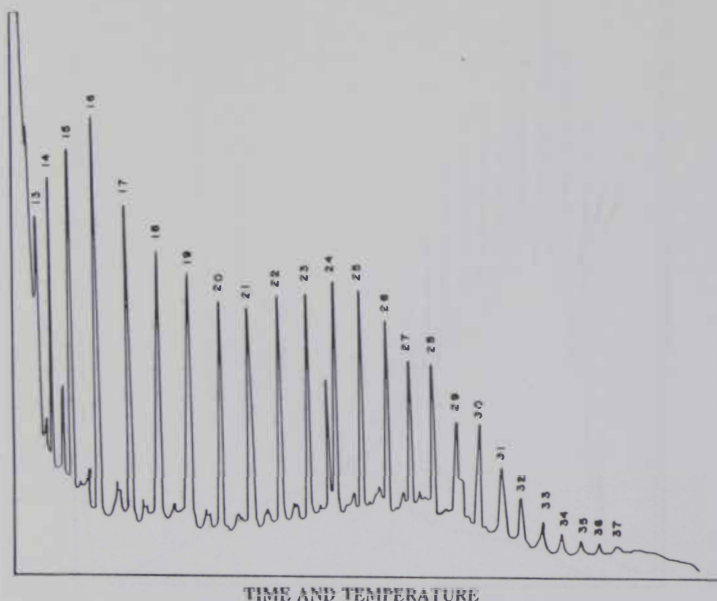


Figure 1. Gas chromatograph of hydrocarbon extract from limestone sample #1 from top of Sacajawea Peak in Bridger Range. Temperature increased during run from 100°C to 300°C @ 10°C/min.

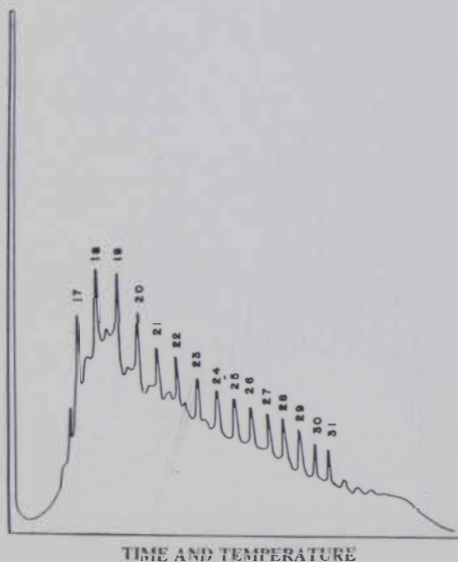


Figure 2. Gas chromatograph of hydrocarbon extract from limestone sample #2 from ridge between Sacajawea's twin peaks. Temperature increased during run from 100°C to 300°C @ 10°C/min.

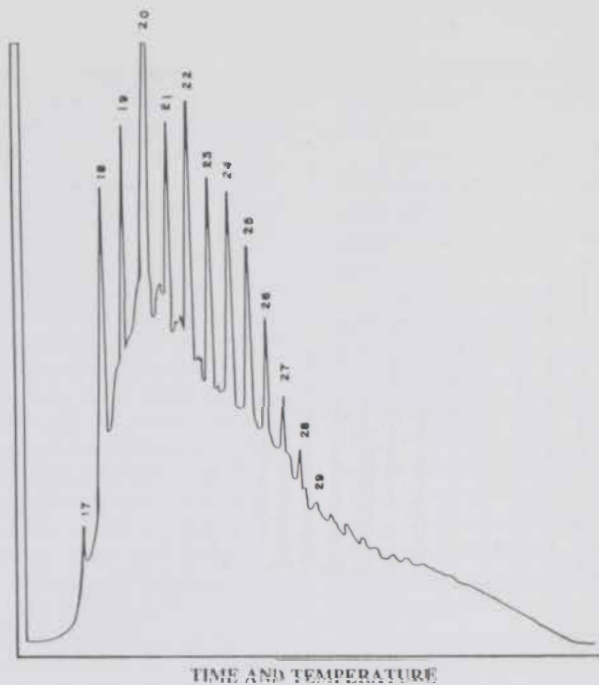


Figure 3. Gas chromatograph of hydrocarbon extract from limestone sample #3 from ridge between Sacajawea's twin peaks. Temperature increased during run from 100°C to 300°C @ 10°C/min.

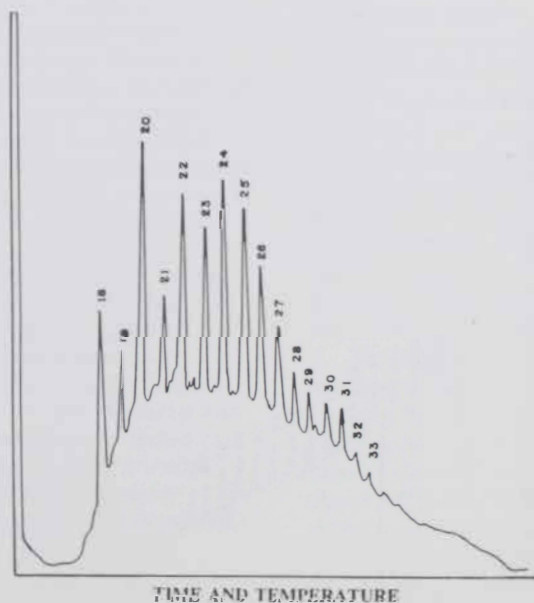


Figure 4. Gas chromatograph of hydrocarbon extract from limestone sample #4 from ridge between Sacajawea's twin peaks. Temperature increased during run from 100°C to 300°C @ 10°C/min.



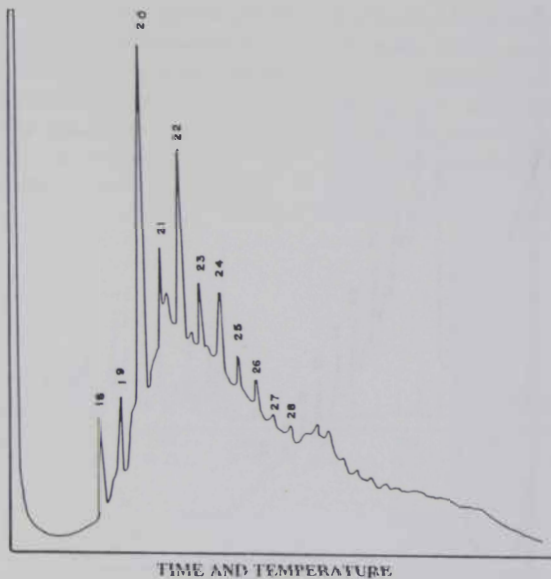


Figure 5. Gas chromatograph of hydrocarbon extract from limestone sample #5 from ridge between Sacajawea's twin peaks. Temperature increased during run from 100°C to 300°C @ 10°C/min.

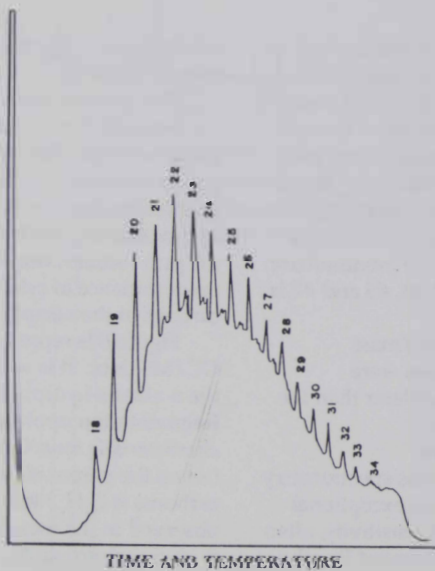


Figure 6. Gas chromatograph of hydrocarbon extract from limestone sample #6 from ridge between Sacajawea's twin peaks. Temperature increased during run from 100°C to 300°C @ 10°C/min.

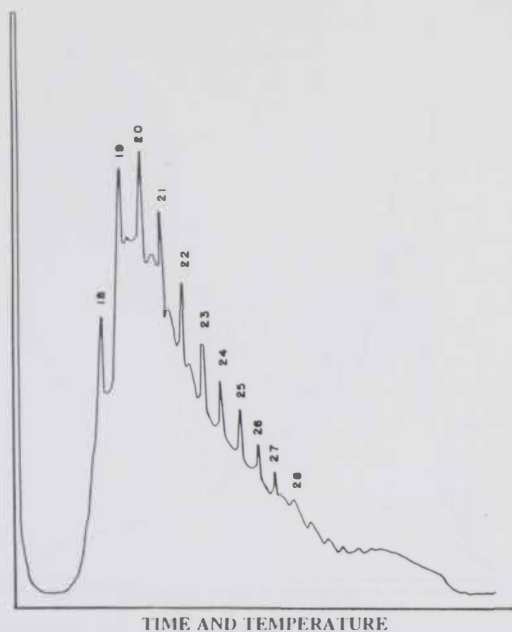


Figure 7. Gas chromatograph of hydrocarbon extract from limestone sample #7 from ridge between Sacajawea's twin peaks. Temperature increased during run from 100°C to 300°C @ 10°C/min.

What we have said about the lack of predominance of the odd numbered carbons in the normal alkanes of sample #1 can be said also of the other samples. In fact, there is a predominance of even numbered carbons for the  $C_{20}$ - $C_{24}$  range in sample #4 and the smaller  $C_{20}$ - $C_{22}$  range in samples #3 and #5. It is also interesting to note that the predominant n-alkane in samples #3, #4, #5 and #7 is  $C_{20}$ .

Gas chromatography/mass spectrometry experiments were conducted some 25 years later than the original chromatographic measurements. Modern chromatography and mass spectrometry instrumentation provides exceptional levels of specificity and sensitivity, often eliminating the requirement of lengthy sample work-up. The differences observed in the recent GC/MS chromatograms, as compared to the

original work, reflect differences in sample work-up.

The analysis of sample #8 was for the purpose of confirmation of the previous data. The high resolving power of capillary chromatography, coupled to the specific identification of hydrocarbon types by MS, confirmed the prior observation of the lack of predominance of odd or even numbered carbons in the normal alkane series.

Figure 8 is representative of the GC/MS data. It is well known that all the n-alkane hydrocarbons undergo fragmentation upon ionization to form characteristic ions. One of these ions (when the parent alkane has six or more carbons) is  $C_6H_{13}^+$  ( $m/z = 85$ ), as observed in the mass spectrum in Figure 8. A chromatographic ion profile can be generated from mass 85 and compared to the original total ion current profile (Fig. 8b vs. Fig. 8a). The direct

comparison of the chromatographic profiles allows a rapid assessment of the identification of the n-alkane series. For example, the component at scan number 2058 (the displayed mass spectrum) has been positively identified as a C<sub>17</sub> n-alkane. The carbon numbers of the remaining n-alkanes in the

chromatogram can be readily determined by adding a carbon to each successive alkane peak encountered while advancing from left to right from the C<sub>17</sub> peak. The n-alkanes in sample #8, ranging from C<sub>13</sub> to C<sub>35</sub>, have been positively identified.

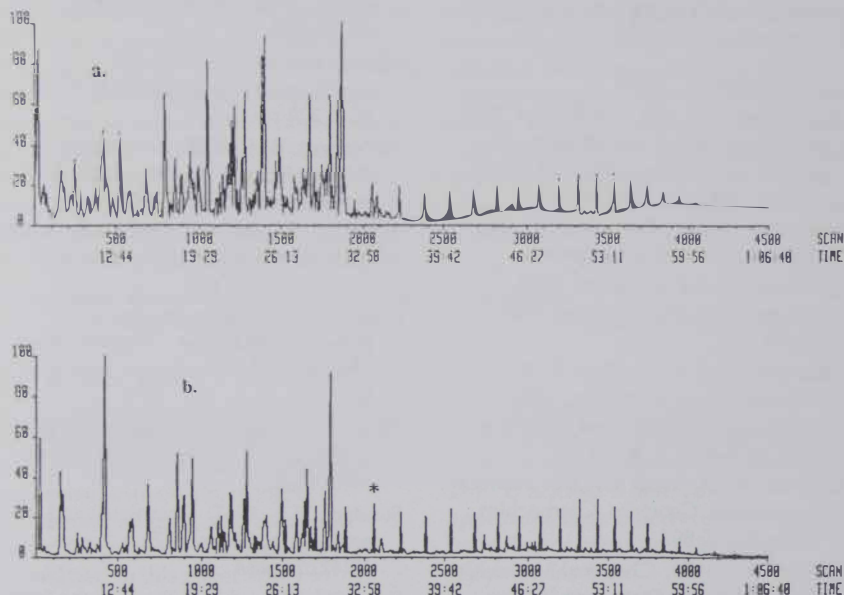


Figure 8. a.) The total ion current chromatogram of concentrate from hexane extraction of sample #8 from top of Sacajawea. b.) The reconstructed ion chromatogram of concentrate from sample #8 showing the distribution of components producing C<sub>6</sub>H<sub>13</sub><sup>+</sup>, an ion of mass 85 and characteristic of n-alkanes have six or more carbons.

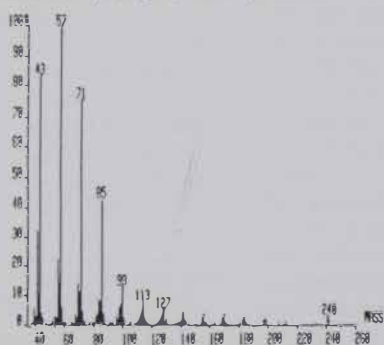


Figure 9. The mass spectrum in the insert is that of the C<sub>17</sub> n-alkanes at scan 2058. (see asterisk in Figure 8b.).



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