Isoprenoid Fatty Acids Isolated from the Kerogen Matrix of the Green River Formation (Eocene)

Abstract. *Demineralized oil shale was oxidized with chromic acid, and C_{14} to C_{22} isoprenoid fatty acids were isolated and identified. The major components of the branched-chain acid fraction after successive 3-, 6-, and 15-hour oxidations are C_{15} and C_{16} branched-chain acids; C_{17}, C_{19} (norphytanic acid), and C_{20} (phytanic acid) were obtained in lower concentration; no C_{18} product was obtained. Separations were effected by column and gas-liquid chromatography, and the structures of individual components were determined by high- and low-resolution mass spectrometry.*

A homologous series of normal fatty acids has been found in the organic acid fraction of the extractables from the Green River Formation (Eocene) (*1*). This fraction includes *iso* and *anteiso* acids (*2*) and isoprenoid fatty acids from C_9 to C_{21}, with the exception of C_{13} and C_{18} (*3, 4*), dicarboxylic acids ranging from C_{12} to C_{18} (*4*), methylketo acids (*5*), and several series of aromatic acids (*6*). The acids isolated from the extract after demineralization of the exhaustively extracted oil shale were essentially the same as those already reported as being present in the solvent-soluble fraction of the shale, although the relative concentrations of individual components differed significantly (*7*). The organic matter in the oil shale is largely in the form of an insoluble polymer (kerogen), and there have been few degradation studies designed to elucidate the molecular structure of the constituents. Exhaustive oxidation of the kerogen with alkaline permanganate has yielded about 96 percent of the organic carbon as water-soluble products, and oxidations under milder conditions have yielded longer-chained (up to C_8) dicarboxylic acids (*8*). Chromic acid oxidation for 6 hours results in a complex mixture of acids (*9*), although only the normal fatty acids ranging from C_{14} to C_{30} were identified. We report here the occurrence of isoprenoid fatty acids, ranging from C_{14} to C_{22} in the branched-chain acid fraction, from the successive 3-, 6-, and 15-hour chromic acid oxidations of Green River Formation kerogen (*10*).

The oil shale sample was obtained from Parachute Creek, 8 miles (13 km) northwest of Grand Valley, Colorado [108°7′W; 39°37′N; elevation 7300 feet (2125 m)]. Pulverization, extraction with a mixture of benzene and methanol (3:1), demineralization with a mixture (1:1) of concentrated HCl and HF, and exhaustive extraction with a benzene-methanol mixture (4:1) made the sample ready for oxidation. The elemental analysis for kerogen was as follows: 65.9 percent C, 8.2 percent H, 0.66 percent N, and 0.90 percent S. Twenty-five grams of kerogen concentrate were refluxed for 3 hours with 3*M* chromic acid in sulfuric acid. The kerogen residue was filtered off, washed with water, and extracted three times with heptane, ultrasonication being used to insure thorough extraction. The spent chromic acid solution was also extracted with heptane, the extracts were combined, and the acids were separated from the neutrals. Esterification with BF_3 in methanol yielded 0.034 g (0.13 percent of the kerogen concentrate) of total esters. The esters in benzene solution were then passed over a silica-gel column (activated with 5 percent H_2O). Normal esters were separated from branched-chain esters by clathration with urea. A urea-heptane clathrate was prepared by dissolving 1 g of urea in enough methanol to make a saturated solution and then adding 0.3 ml of *n*-heptane containing 1 percent *n*-decane. The esters in 2 ml of benzene were allowed to equilibrate for 20 hours with the urea-heptane adduct. After being cooled, the urea–*n*-ester adduct was filtered off, washed with cold heptane, and destroyed with water; the yield of normal esters, which were extracted with heptane, was 0.016 g (0.06 percent). The branched-chain esters extracted from the adduct solution amounted to 0.010 g (0.04 percent). The residual kerogen was further oxidized for an additional 6 hours, the yield being 0.009 g (0.04 percent) of normal and 0.005 g (0.02 percent) of branched-chain acid fraction. The kerogen residue remaining from the 6-hour oxidation was oxidized for an additional 15 hours; the yield was 0.012 g (0.05 percent) of normal and 0.008 g (0.03 percent) of branched-chain acid fraction. All branched-chain acid fractions were chromatographed on a column (1.5 m by 3 mm), packed with 3 percent SE-30 on chromosorb and programmed from 100° to 250°C at 10° per minute with a flow rate of 40 ml/min (Fig. 1).

High-resolution mass spectrometry of the three esterified branched-chain fractions showed molecular-ion compositions corresponding to acids ranging from C_5 to C_{26} with a primary mode at C_{16}. The presence of esters of $\leq C_4$ cannot be determined definitely from a high-resolution mass spectrum per se, since the McLafferty rearrangement of the ester function can give rise to even mass peaks, that is $C_3H_6O_2$, $C_4H_8O_2$, and the peak due to rearrangement for any free acids present would be $C_2H_6O_2$.

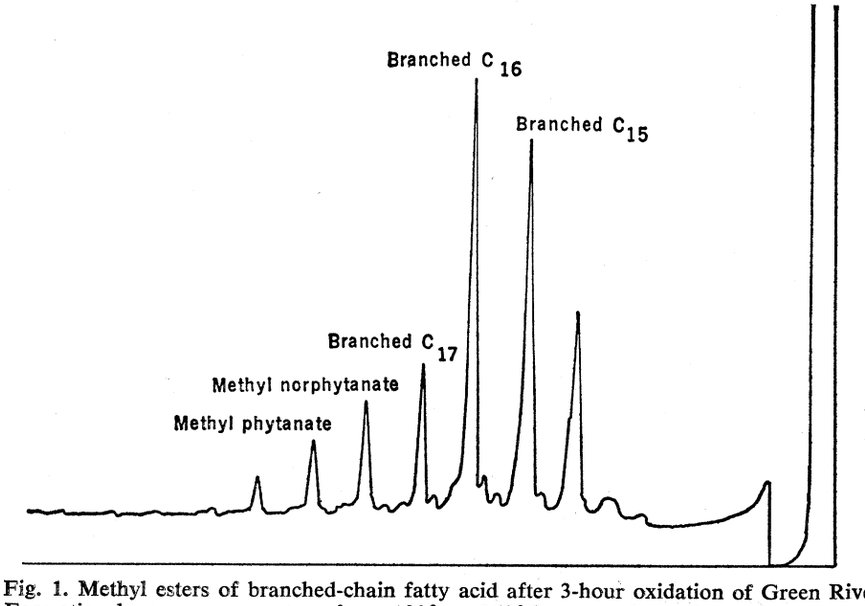

Fig. 1. Methyl esters of branched-chain fatty acid after 3-hour oxidation of Green River Formation kerogen; temperature from 100° to 250°C on a column 1.5 m by 3 mm, 3 percent SE-30.

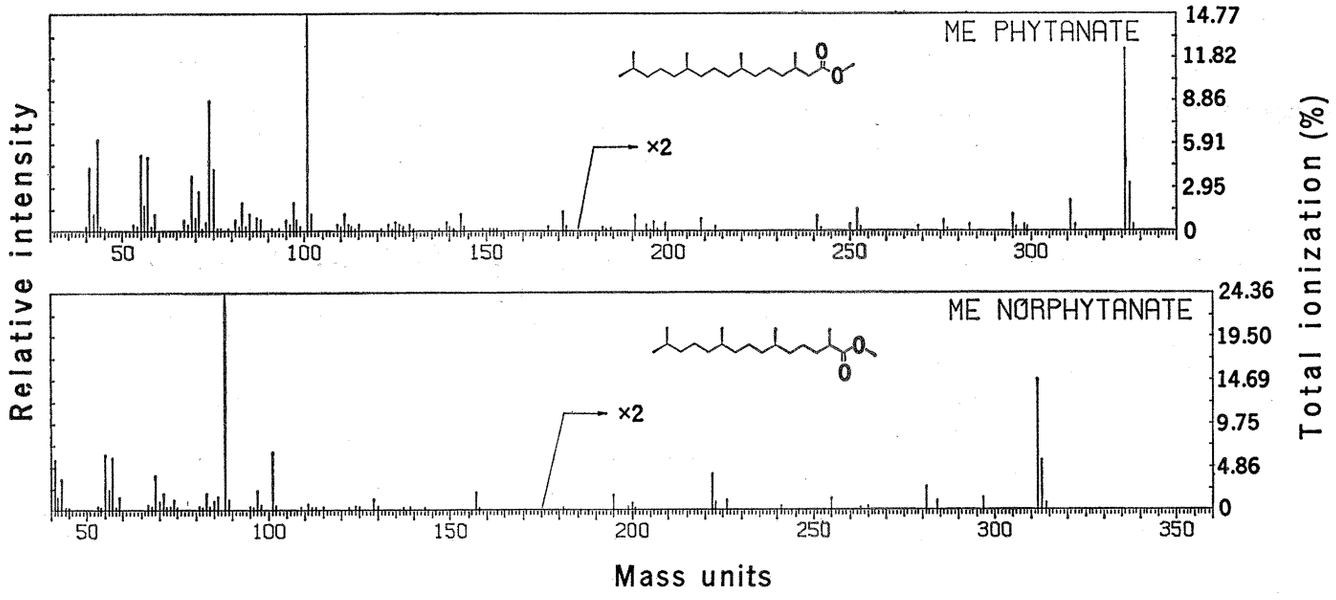

Fig. 2. Low-resolution mass spectra of methyl phytanate and methyl norphytanate isolated from the chromic acid oxidation products of Green River Formation kerogen.

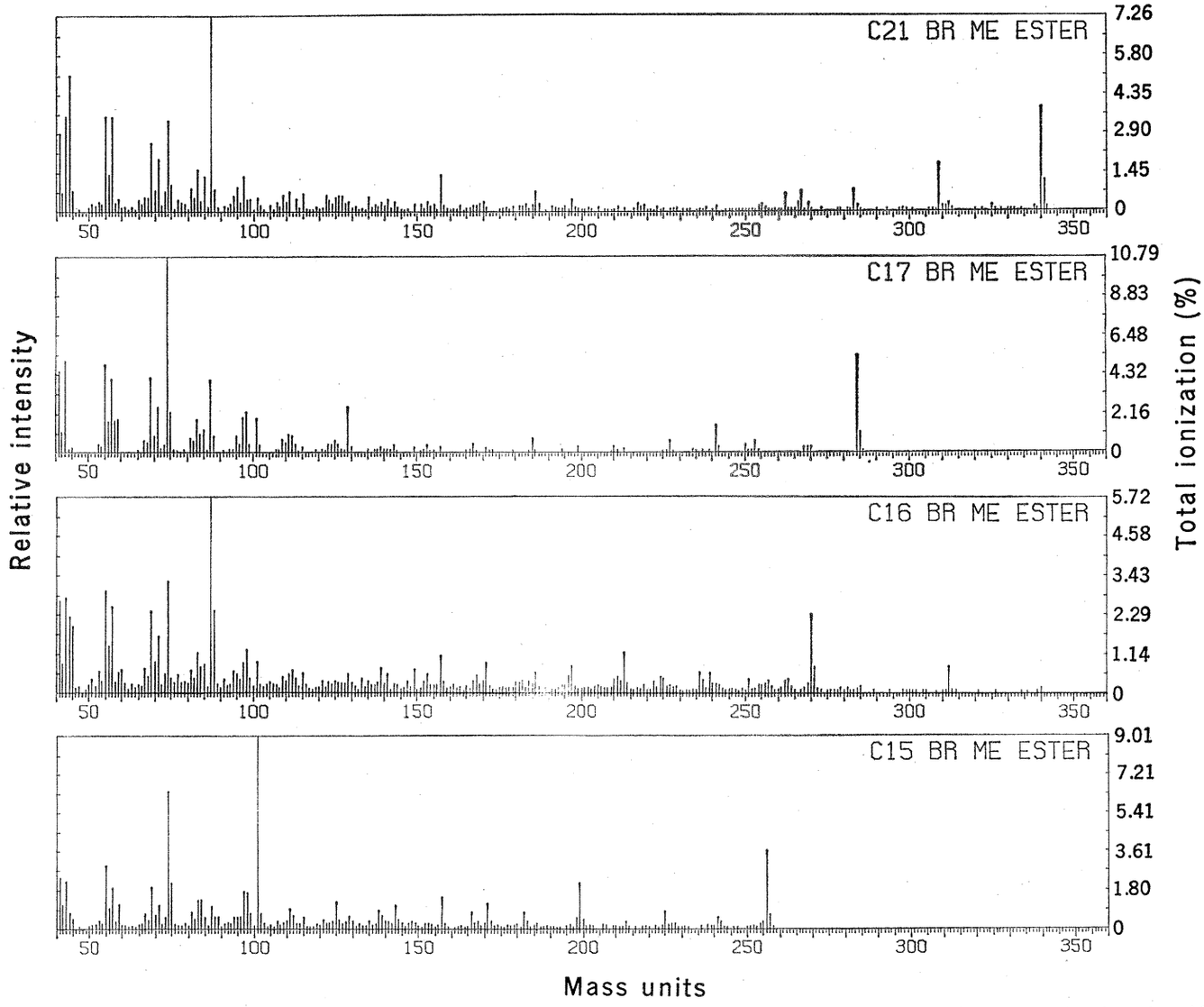

Fig. 3. Low-resolution mass spectra of the C_{15}, C_{16}, C_{17}, and C_{21} branched-chain acid methyl esters isolated from the chromic acid oxidation products of Green River Formation kerogen.

The molecular ions of the C_9 and C_{13} esters are of very low relative abundance; the C_{19} and C_{24} are entirely absent. The major peaks in the gas-liquid chromatograms of the esters ranged from C_{15} to C_{23} with a maximum also at C_{17}. There is a large peak due to the C_{16} acid for both the high-resolution mass spectrum and the gas-liquid chromatogram of the branched esters. The major peaks were also collected, and their low-resolution mass spectra were determined. The following acids were identified from the mass spectra of their methyl esters (Figs. 2 and 3): 3,7,11-trimethyldodecanoic acid; 4,8,12-trimethyltridecanoic acid; 5,9,13-trimethyltetradecanoic acid; 2,6,-10,14-tetramethylpentadecanoic acid (norphytanic acid); 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid); and 4,8,12,16-tetramethylheptadecanoic acid. Coinjection of methyl phytanate enhanced its respective peak in the analytical gas-liquid chromatograms.

The aliphatic isoprenoid carbon skeleton has been isolated from carbonaceous sediments of various geologic formations ranging from the early Precambrian to the most recent (*11*), and has been taken as presumptive evidence for the occurrence of the photosynthetic process as far back in terrestrial chronology as 3.2 billion years. The possibility of migration of organic molecules after sediment deposition and compaction has brought up in previous discussions the question whether these molecules are indeed indigenous. McCarthy and Calvin (*12*) have discussed the significance of the molecular structures of hydrocarbons with respect to their possible diagenetic transformations and biological or nonbiological origin. The isolation and identification of any particular molecular species is of debatable significance without the assurance that the species was laid down at the time of deposition and compaction of the sediment.

The occurrence of isoprenoid acids in the extract from the chromic acid oxidation of kerogen concentrate from the Green River Formation is evidence that these acids are bound to the kerogen matrix. This means that they are indigenous and were laid down at the time of sedimentation and thus incorporated into polymeric organic structures. The nature of their linkage to the matrix is still under investigation at this laboratory. It is therefore of fundamental significance to corroborate earlier findings with this evidence. In addition, a knowledge of a particular molecule's structure and abundance, relative to the large number of organic components present in any carbonaceous rock, must be ascertained before formulating hypotheses regarding that particular molecule's diagenesis and significance as a "biological marker." For such reasons, detailed characterization of the acidic components of the solvent extracts of the total organic matter in the Green River Formation has been carried out (*3, 4, 5, 6, 13*). Such findings should catalyze similar studies of carbonaceous sediments as a function of chronologic age.

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Asparagine Synthetase Activity of Mouse Leukemias

Abstract. *Various transplanted leukemias and normal tissues of the mouse were tested for asparagine synthetase activity. Leukemias susceptible to suppression by asparaginase have little or no synthetase activity. In contrast, leukemias insensitive to asparaginase exhibit substantial and often very high asparagine synthetase activity. Asparaginase-resistant variants of sensitive leukemias also have considerable synthetase activity. Thus the requirement by certain malignant cells of exogenous asparagine, which entails sensitivity to asparaginase, may be ascribed to lack of asparagine synthetase. Development of asparaginase-resistant variants from asparaginase-sensitive lines is consistently associated with acquisition of asparagine synthetase activity.*

Kidd's observation (*1*) that administration of guinea pig serum causes regression of certain mouse and rat lymphomas has been confirmed (*2*, *3*). Broome produced evidence that the antitumor activity of guinea pig serum is due to its content of L-asparaginase (*4*); this also has been extensively confirmed (*5*). Thus asparaginase of *Escherichia coli* was shown (*6*) to have an antitumor effect similar to that of asparaginase of guinea pig serum. Lymphoma in the dog (*7*, *8*) and lymphoblastic leukemias in man (*8*, *9*) may respond to therapy with asparaginase. The evidence points to the conclusion that tumors and leukemias that respond to asparaginase depend on an external supply of asparagine, and that when this is hydrolyzed by asparaginase in vitro or in vivo the cells die.

A plausible explanation of the requirement for asparagine by certain malignant cells is that they have lost the capacity to synthesize it. Several pathways of biosynthesis of asparagine have been proposed (*10*). Conversion of aspartate to asparagine in certain microorganisms has been shown to require ammonia and adenosine 5′-triphosphate (ATP) and to be associated with cleavage of ATP to inorganic pyrophosphate and adenosine 5′-monophosphate (*11*). The amide nitrogen atom of glutamine rather than the nitrogen of ammonia is utilized for asparagine synthesis in HeLa cells (*12*), and subsequent work (*13*, *14*) has confirmed the participation of glutamine in the synthesis in vitro of asparagine in mammalian systems. Synthesis of asparagine by preparations of Jensen sarcoma and from nutritional