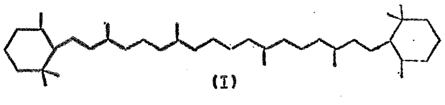
(1957). For a comparison with wings of ants and Plumariidae, see W. L. Brown, Jr., and W. L. Nutting, *Trans. Amer. Entomol. Soc.* **75**, 113 (1950).
4. F. M. Carpenter, J. W. Folsom, E. O. Essig, A. C. Kinsey, C. T. Brues, M. W. Boesel, H. E. Ewing, *Univ. Toronto Stud. Geol. Ser.* **40**, 7 (1934).
5. We gratefully acknowledge the splendid cooperation of Mr. and Mrs. Frey, as well as the intermediary aid of Dr. Donald Baird of Princeton University and Mr. David Stager of the Newark Museum.
6. W. L. Brown, Jr., *Insectes Sociaux* **1**, 21 (1954).
7. We acknowledge the aid of H. E. Evans, who gave us the benefit of extensive comparisons of *Sphecomyrma* characters with those of various wasp genera. In classifying tiphiids, we have arbitrarily followed the system of V. S. L. Pate, *J. N.Y. Entomol. Soc.* **55**, 115 (1947).
8. E. O. Wilson, F. M. Carpenter, W. L. Brown, Jr., *Psyche*, in press.

26 June 1967

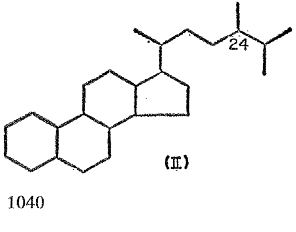
Perhydro-β-Carotene in the Green River Shale

Abstract. *Several triterpenes and the tetraterpene perhydro-β-carotene have been identified in the branched-cyclic hydrocarbon fraction of this Eocene shale. The analytical procedure included thiourea adduction followed by combined gas chromatography and mass spectrometry.*

The identification of an optically active triterpene, gammacerane, in the Green River Oil Shale bitumen (*1*) and the isolation of three pentacyclic—but as yet unidentified—triterpenes from an optically active petroleum distillate from a Nigerian crude oil (*2*) have provided further evidence for the biogenesis of some of the organic matter indigenous to these geological situations. Similar chemical markers are the acyclic isoprenoid hydrocarbons, phytane and pristane, along with their corresponding acids, reported for the Green River shale (*3, 4*) and a California petroleum (*5*). The presence of these compounds is significant because they appear to be stable geologically, and because the developed analytical methods are now available for other specimens such as Precambrian sediments, meteorites, and returned lunar samples.



(I)



(II)

The Green River Formation is known to have suffered little from geological stresses such as temperature, pressure, and fracture (*6*). From this richly organic deposit resulting from fresh water lakes of Eocene age (50 to 60 million years old), we have now identified perhydro-β-carotene, $C_{40}H_{78}$ (I). This is the first time that a tetraterpene has been isolated from a geological sample, though sesqui-, di-, and triterpenes have been identified in this and other sediments (*7*). Thus the presence of a tetraterpene completes the typical isoprenoid pattern found in plants today. In fact, almost all of the peaks observed in the gas chromatograms of the Green River shale alkanes are accountable as hydrocarbons of obvious biological origin. The precise history and the apparent stability of these hydrocarbons under geological conditions require further study.

A sample of Green River shale (600 g) from Rifle, Colorado, (*8*) was crushed to 1-cm (about) chunks and cleaned with a mixture of benzene and methanol (1 : 1). After being dried in air, the rock was pulverized to pass 100 mesh and then extracted in 50-g amounts with *n*-hexane (200 ml). Three extractions with fresh solvent were completed for each batch of rock. The suspensions were subjected to ultrasonic vibration for 20 minutes, each time, before the mixture was centrifuged to permit decanting of the solvent. After the hexane was evaporated the extracted organic matter (5.3 g) was chromatographed on an alumina column (85 by 5 cm), and the hydrocarbon fraction was eluted with *n*-hexane (1 liter). The first 600 ml contained the desired hydrocarbons, as monitored by thin-layer chromatography (silica gel G, hexane developed). This fraction was dissolved in isooctane and passed through a 5-Å molecular sieve (Linde, activated at 320°C under a stream of dry N_2 for 24 hours) to remove the normal alkanes. The branched-cyclic alkanes (1.4 g) remaining in the isooctane were further separated by formation of adduct with thiourea (*9*). For formation of adducts, alkanes (10 mg) were dissolved in chloroform (1 ml) in a Craigee tube. A saturated solution of thiourea in methanol (1 ml) was added, the mixture was heated until the solution was homogeneous, cooled, and held at room temperature for 24 hours; during this time adduct crystals formed. In appearance, the hexagonal adduct needles were distinct from the usual tetragonal thiourea crystals (*10*). The needles were collected, washed twice with cold chloroform, and then dissolved in hot water to release the trapped (adduct) hydrocarbons. The ratio of adduct to nonadduct hydrocarbons in the branched-chain cyclic fraction was 1:7.

The gas chromatograms of the adduct and nonadduct fractions are presented in Fig. 1. The acyclic isoprenoid hydrocarbons reported previously (*3*) are adducted almost completely, but rather unexpectedly some cyclic isoprenoids are also included by thiourea. Both fractions were then separated further by preparative gas-liquid chromatography (3 percent SE-30, 6-m by 6-mm column). Repeated collection of single chromatographic peaks in capillary tubes provided sufficient sample for infrared spectroscopy (micro) and combined gas chromatography–mass spectrometry (GCMS). Several significant peaks in the triterpene region were analyzed in the LKB-9000 gas chromatograph–mass spectrometer (1 percent SE-30, 3-m by 3-mm column). Cholestane (II, R=H), ergostane (II, $R=CH_3$), and sitostane (II, $R=C_2H_5$) were identified in the adduct fraction. Ergostane is present in the largest amount. However, most of the sitostane remains in the nonadduct, and accounts for the component present in the largest amount in the triterpenes isolated from the shale. Further identification of these components awaits extended purification and comparison with standard samples, many of which are not available at the present time.

Before this investigation, substituted naphthalenes and anthracenes were the largest molecules known to be included by the thiourea channel (*11*). The adduction of cholestane under the same experimental conditions as the adduction of acyclic isoprenoids has been confirmed by x-ray analysis (*12*). The adduct crystal of cholestane shows predominantly the same reflections as does that of the pristane adduct, differing thereby from the thiourea crystal. The weak intensities of the reflections from the organic guest molecules compared with those from the thiourea presently preclude the determination of the way in which the cholestane molecule is included in the thiourea channel (*12*). Cholestane, ergostane, and sitostane differ only in the substitution on carbon No. 24, in that they have an H, a methyl, and an ethyl group in this position respectively (II). Curiously, coprostane and 3β-bromo-3α-methylcholestane also formed adducts with thiourea, but other halogen-substituted cholestanes did not form adducts under the same conditions. Androstane, with no side chain, does not form adducts with thiourea. In a 1:1 mixture of cholestane and pristane, 95 percent of the cholestane and only 90 percent of the pristane form adducts with thiourea under the conditions described. Treatment of the filtrate with a second portion of thiourea does not yield more adduct, but a 100-percent yield of adduct is obtained by evaporation of the original solution. However, the crystalline mass is then difficult to wash.

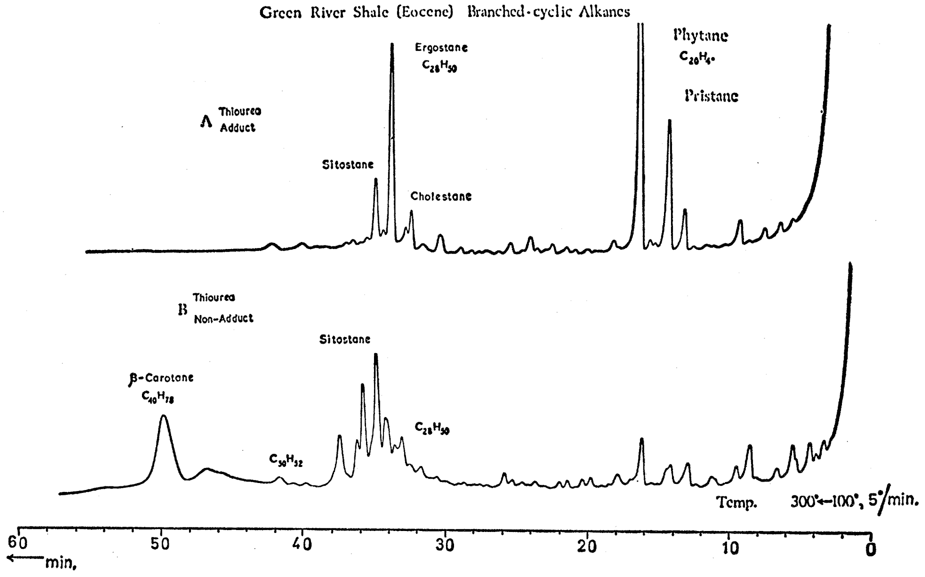
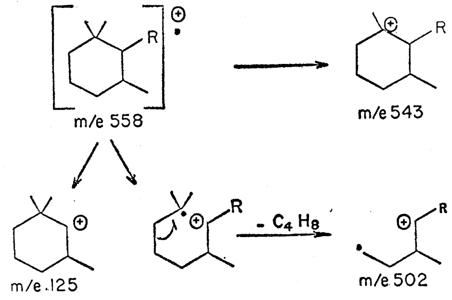


Fig. 1. Gas-liquid chromatograms of (A) the adduct and (B) nonadduct portions of the branched-cyclic hydrocarbon fraction from the Green River shale. Conditions: 1.5-m by 2-mm column of 4 percent silicone rubber JXR on Chromosorb G. Temperature programed from 100° to 300°C at 5° per minute.

Perhaps the most significant component in the branched-cyclic alkanes of the Green River shale is the high-molecular weight compound that appears on the gas chromatogram and has a retention time longer than those of the triterpenes. This component, present as a major constituent of this branched-chain cyclic fraction, remained in the nonadduct fraction. It was separated from the nonadduct fraction by preparative gas-liquid chromatography and was then subjected to GCMS where several minor (< 1 percent) peaks were observed on the analytical column. A mass spectrum [scan 4.5 seconds, *m/e* (mass/charge) range 20 to 600] taken during the elution of the major peak showed that it was derived mainly from a compound of molecular weight 558, corresponding to the formula $C_{40}H_{78}$. However, the spectrum also showed minor impurities at (M-2), (M-4), and so on up to (M-12). The sample was therefore purified by Ag^+-silica thin-layer chromatography. The mass spectrum of the resulting colorless oil is reproduced in Fig. 2. Although some impurities had been removed, peaks at (M-2) and so on still persisted, although they were diminished in intensity. The amount of impurities may be very small because an increasing number of double bonds or rings gives greater stability to the parent ion in the mass spectrometer (*13*). The abundant ions at *m/e* 543, 502, and 125 suggested that the major constituent had the perhydro-β-carotene structure (*14*).



However, interpretation of the mass spectrum of a highly branched saturated hydrocarbon can give only partial identification of the carbon skeleton and accordingly an authentic sample of perhydro-β-carotene was prepared by hydrogenation of β-carotene (*15*). The spectrum of this is also reproduced in Fig. 2 and is almost identical to that of the Green River compound, minor differences being explicable by the presence in the latter of the above-mentioned impurities. Perhydro-β-carotene as initially prepared did have unsaturated contaminants, but these were easily removed by Ag^+-silica thin-layer chromatography, by precisely the same procedure as used for the Green River hydrocarbon.

Further attempts to purify the Green River hydrocarbon by gas-liquid chromatography over various phases and by treatment with bromine followed by Ag^+-silica thin-layer chromatography were unsuccessful. Accordingly, we suggest that the impurity peaks in the spectrum (Fig. 2) of this sample are due to the presence of hydrocarbons with additional rings. We did obtain, eventually, a simplified mass spectrum by introducing a relatively large sample (about 5 μg) into the mass spectrometer by means of the direct insertion probe and then allowing the sample to be pumped away slowly. The impurity peaks (for example, at *m/e* 554, 552, 550, 548) were virtually absent, and the final spectrum was indistinguishable from that (Fig. 2) of authentic perhydro-β-carotene.

The infrared spectra for the geological and the authentic samples (Fig. 3) show good correspondence, and the minor differences many be accounted for by the impurities mentioned above and the stereoisomeric possibilities inherent in structure I. The bands, γ (CH_3) symmetrical, near 1385 and 1365 cm^{-1} demonstrate the presence of the *gem*-dimethyl groups in addition to the in-chain methyls (1375 cm^{-1}). Further confirmation of the identity of the two samples of C_{40} alkane comes from the nuclear magnetic resonance spectra (small sample sizes precluded high quality spectra) and measurement of the Kovats indices (*16*). These were 3694 and 3690 for the geological and authentic samples, respectively, when measured on SE-30 at 270°C. Coinjection of the authentic β-carotene and the geological sample resulted in a single peak on the chromatogram.

The identification of perhydro-β-carotene in the Green River shale and the further definition of the triterpenes present provides additional evidence for a prominent contribution from the plant growth of that time. Perhydro-β-carotene is not known as a plant constituent but β-carotene, the expected precursor of the saturated hydrocarbon, is itself very labile and readily oxidized. Any oxidizing environment must have been of short duration. Reduction, presumably during diagenesis of the sediment, must have been rapid for the carbon skeleton to be preserved. Indeed, Bradley has remarked on the low oxygen content of the organic matter in the shale extracts (*17*). Our results warrant continued search for unsaturated tri- and tetraterpenes—for example, β-carotene itself as well as oxygenated derivatives.

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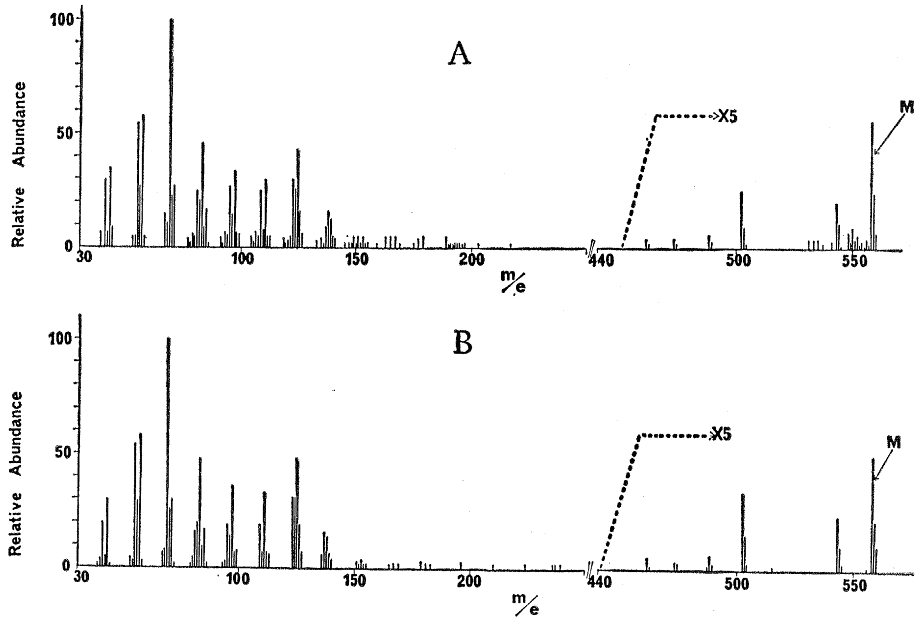

Fig. 2. Mass spectra of $C_{40}H_{78}$ hydrocarbons. (A) Hydrocarbon from Green River shale, partially purified by gas chromatography and preparative (Ag^+-silica) thin-layer chromatography. (B) Authentic perhydro-β-carotene. Recording conditions: LKB-9000 mass spectrometer; 70 ev; direct insertion probe; evaporation temperature 125°; ion-source temperature 300°C.

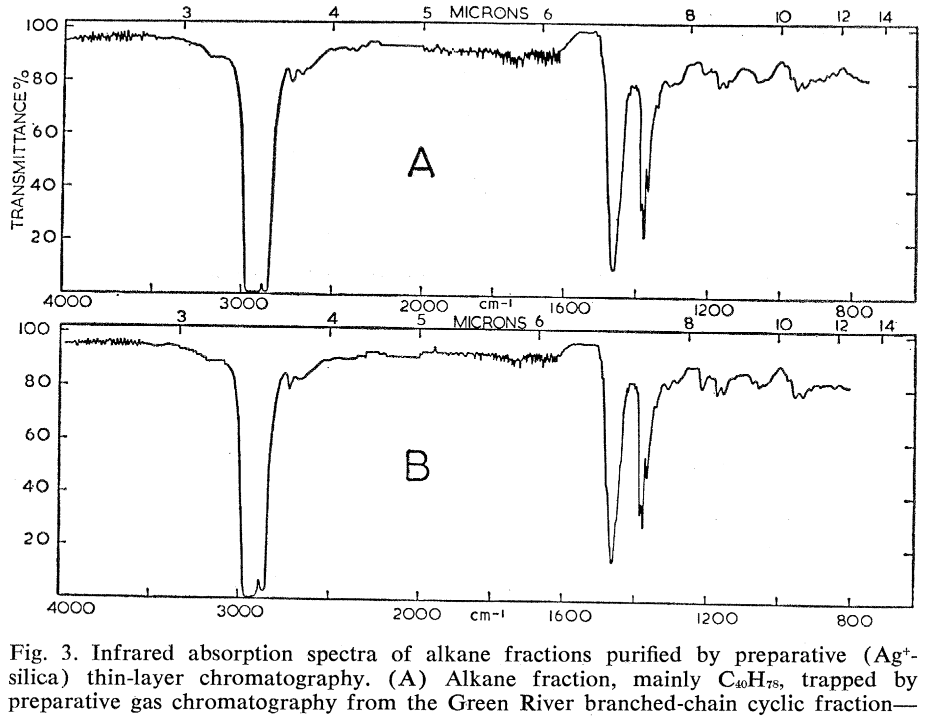

Fig. 3. Infrared absorption spectra of alkane fractions purified by preparative (Ag^+-silica) thin-layer chromatography. (A) Alkane fraction, mainly $C_{40}H_{78}$, trapped by preparative gas chromatography from the Green River branched-chain cyclic fraction—thiourea nonadduct. (B) Perhydro-β-carotene obtained by catalytic reduction of pure β-carotene (*15*). Recording conditions: Perkin-Elmer model 257. A solution of the alkane fraction (about 100 μg) dissolved in carbon tetrachloride (3 μl) was injected into a "microscope cell" [0.5 mm (*18*)] and examined with a beam condenser [× 4 (*18*)] Scanning time: 11 minutes. The reference beam was appropriately attenuated and contained a cell of variable path length (CCl_4). The fine structure near 3700 and 1700 cm^{-1} is due to water vapor absorption.

References and Notes

1. I. R. Hills, E. V. Whitehead, D. E. Anders, J. J. Cummins, W. E. Robinson, *Chem. Commun.* 752 (1966).
2. I. R. Hills and E. V. Whitehead, *Nature* **209**, 977 (1966).
3. A. G. Douglas and G. Eglinton, *Comparative Phytochemistry* (Academic Press, London and New York, 1966), pp. 57–77.
4. G. Eglinton, A. G. Douglas, J. R. Maxwell, J. N. Ramsay, S. Stallberg-Stenhagen, *Science* **153**, 1133 (1966).
5. J. Cason and D. W. Graham, *Tetrahedron* **21**, 471 (1965).
6. N. de Nevers, *Sci. Amer.* **214**, 21 (1966); B. Shaeffer and M. Mangus, *Natur. Hist.* **74**, 10–21 (1965); W. H. Bradley, *Amer. J. Sci.* **262**, 413 (1964); W. E. Robinson, J. J. Cummins, G. V. Dineen, *Geochim. Cosmochim. Acta* **29**, 249 (1965); G. Eglinton, P. M. Scott, T. Belsky, A. L. Burlingame, M. Calvin, *Science* **145**, 263 (1964); W. E. Robinson, D. L. Lawlor, J. J. Cummins, J. I. Fester, *U.S. Bur. Mines Rept. Invest. No. 6166* (1963); J. J. Cummins and W. E. Robinson, *Chem. Eng. Data* **9**, 304 (1964).
7. A. Burlingame, P. Haug, T. Belsky, M. Calvin, *Proc. Nat. Acad. Sci. U.S.* **54**, 1406 (1965); B. J. Mair, Z. Ronen, E. J. Eisenbraun, A. G. Horodysky, *Science* **154**, 1339 (1966).
8. Bureau of Mines, Laramie, Wyo.
9. M. Baron, in *Physical Methods in Analytical Chemistry*, W. Berl, Ed. (Academic Press, New York, 1961), vol. 4, pp. 226-32.
10. N. Nicolaides and F. Laves, *J. Amer. Oil Chem. Soc.* **40**, 400 (1963).
11. D. Montgomery, *J. Chem. Eng. Data* **8**, 433 (1963); A. Van der Wiel, *Erdoel Kohle* **18**, 632 (1965).
12. A. Kerr, private communication (1966).
13. K. Biemann, *Mass Spectrometry* (McGraw-Hill, New York, 1962), p. 81.
14. The prefix β- is used to indicate structure (I) for the geological alkane; α- or β-carotene, a less saturated analog, or even oxygenated derivatives could have provided the carbon skeleton prior to hydrogenation. The stereochemistry for the various methyl substituents is not known.
15. Purified and stored under nitrogen by Dr. J. A. Blair of the University of Strathclyde.
16. E. Kovats, *Helv. Chim. Acta* **41**, 1915 (1958).
17. W. H. Bradley, private communication (1967).
18. Research and Industrial Instruments Co., Great Britain.
19. We thank Dr. W. E. Robinson (Bureau of Mines, Laramie, Wyo.), for the Green River shale, Dr. J. A. Blair for the samples of β-carotene, and E. V. Whitehead for a sample of perhydro-β-carotene. We thank NASA (subcontract NsG 101-61), the Science Research Council, the Natural Environment Research Council, and Shell International for financial support. One of us (M.T.J.M.) thanks St. Joseph College, Conn., for leave of absence for this research. Tentative identification of perhydro-β-carotene in Green River Formation Oil Shale was reported at the annual meeting of the Geochemical Society, San Francisco, Calif., Nov. 1966. W. E. Robinson and D. E. Anders of the Bureau of Mines, Laramie Research Center, Laramie, Wyo., reported at this meeting an independent tentative identification of the same perhydro-β-carotene in the Green River Oil Shale.

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