Branched Alkanes from Blue–Green Algae

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Summary The mixture of branched alkanes from blue–green algae was found to be 90% 7- and 8-methylheptadecane (1 : 1), and 10% 6-methylheptadecane.

THE hydrocarbon composition of the blue–green algae is apparently very simple.[1–3] The pattern shows normal hydrocarbons in the C_{15}—C_{20} range with a predominance of n-C_{17}. The isoprenoid hydrocarbons, pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) are absent in algae, but are generally present in higher plans and animals.

Methyl-branched alkanes seemed to be of particular significance because the methyl branches are not iso- and anteiso-structures. They appear to occur uniquely in blue–green algae and are absent from photosynthetic and non-photosynthetic bacteria.[4] These hydrocarbons have not been found in the green algae,[2,4] which are more advanced than blue–green algae, but are still relatively primitive.

Instrumental analyses and synthesis of 7,9-dimethylhexadecane and of 7- and 8-methylheptadecanes proved that the branched hydrocarbon first found consisted of a mixture (1 : 1) of 7- and 8-methylheptadecane, which previously could not be separated by g.l.c.[2] The incubation of [*Me*-^{14}C]-L-methionine and tritiated *cis*-vaccenic acid (*cis*-Δ^{11}-C_{18} acid) to form 7- and 8-methylheptadecane indicated that the methionine methyl group was specifically added to the double bond of vaccenic acid. This was followed by decarboxylation to yield the 7- and 8-methylheptadecane.[5]

We now describe a further identification and optical activity measurement of the branched-C_{18} hydrocarbons from blue–green algae. The dried cells (*Nostoc muscorum*) were treated with benzene–methanol (3 : 1). The extracted material was transferred to the top of an alumina column

† Nonylphenoxypoly(ethyleneoxy)ethanol, from General Aniline and Film Corporation, New York, N.Y.

previously washed with n-heptane; it was then eluted with n-heptane, benzene, and methanol. The first fraction (n-heptane fraction) containing the total aliphatic hydrocarbons, was used for the following analyses. The total aliphatic hydrocarbon fraction (2 μg) was analysed on an Aerograph Model 204 gas chromatograph equipped with a high resolution capillary column and flame ionization detector. A high resolution g.l.c. column made of stainless steel, 750 ft., 0·02 in. inside diameter, was used. It was coated with 5% Igepal CO-880† and GE SF-96‡ (1:1) in ether–acetone. The column had efficiencies in excess of 350,000 theoretical plates for branched C_{18} peaks eluting after 12 h.

The branched C_{18} hydrocarbon mixture was separated into equal amounts of 7-methylheptadecane (Figure, peak b) and 8-methylheptadecane (Figure, peak a). However, approximately 10% of peak c was also separated by this high resolution g.l.c. Peak c was identified as 6-methylheptadecane by its mass spectrum. The mass spectrum was taken using a combination of an Aerograph 204 gas chromatograph and an A.E.I. MS-12 mass spectrometer.

For the determination of optical activity 5 mg of total branched-C_{18} hydrocarbons were isolated from the total aliphatic hydrocarbon fraction obtained from 30 g of dried cells by preparative g.l.c. on an Aerograph Model A-90-P. The total hydrocarbon fraction was chromatographed on a temperature-programmed 25 ft. by 0·25 in. 3% SE-30 column. The optical activity was studied on an ETL-NPL Automatic Polarimeter Type 143A, with the sodium D line, 10 ohm load, 5 cm cell length, and 90 mv scale: $[\alpha]_D^{20} \pm 2{\cdot}5 \pm 0{\cdot}5°$ (hexane).

We therefore believe that the enzymic methylation of *cis*-vaccenic acid was a stereospecific reaction. It is possible that the methyl group could be added stereospecifically on the open side of the double bond. The methylation reaction was followed by decarboxylation to yield the optically active branched-C_{18} hydrocarbons.

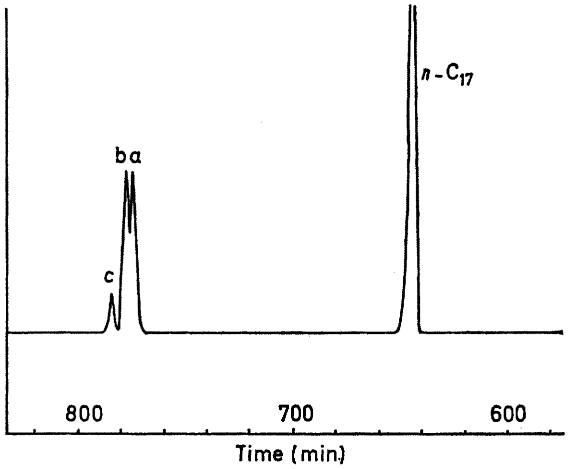

FIGURE. *Gas chromatographic separation of branched-*C_{18} *hydrocarbons. Helium pressure* 30 *lb./in*², *no split. Isothermal at* 125°. *Peaks a, b, and c were identified as* 8-*methylheptadecane,* 7-*methylheptadecane, and* 6-*methylheptadecane, respectively, by g.c.–m.s.*

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‡ Methylsilicone fluid, from the General Electric Company, Waterford, N.Y.

[1] J. Han, E. D. McCarthy, W. VanHoeven, M. Calvin, and W. H. Bradley, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **59**, 29.
[2] J. Han, E. D. McCarthy, M. Calvin, and M. H. Benn, *J. Chem. Soc.* (*C*), 1968, 2785.
[3] P. L. Park, C. Van Baalen, and L. Maurer, *Science*, 1967, **155**, 707.
[4] J. Han and M. Calvin, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **64**, 436.
[5] J. Han, H. W.-S. Chan, and M. Calvin, *J. Amer. Chem. Soc.*, 1969, **91**, 5156.