Formation of Hydrocarbons by Micro-organisms

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1 Introduction

Alkanes and alkenes are a relatively neglected group of natural products; indeed, apart from the obligatory reference to methane in marsh gas, most standard chemistry textbooks make no reference to their biological origin. In large measure this probably reflects the difficulties in separating and identifying individual members of hydrocarbon mixtures prior to the advent of gas–liquid chromatography (g.l.c.). Studies on the biosynthesis of alkanes are also hampered by the difficulty of locating the position of isotopic labels in the carbon chain. A large portion of the work on the biological occurrence and synthesis of hydrocarbons refers to plants[1] and arises partly because material on which to work is relatively easy to obtain and partly because hydrocarbons might be used in phylogenetics. However, over the past few years the production of hydrocarbons by micro-organisms has begun to receive increasing attention for reasons ranging from the recognition of ethylene as a plant hormone to the use of steranes as biological markers in ancient shales.

From an experimental viewpoint the biggest continuing problem is the exclusion of adventitious hydrocarbon (gaseous, liquid, and solid) contamination. This necessitates scrupulous attention to the purity of all solvents, materials, and apparatus used in such studies. An interesting side-light on this problem is the recent suggestion,[2] that the increasingly popular 'parafilm' can be used as an excellent reference source of alkanes for g.l.c. These problems are accentuated by the relatively low hydrocarbon content of micro-organisms, which necessitates growing them on a substantial scale. An unambiguous way of establishing the microbial origin of isolated hydrocarbons is to employ a ^{14}C-labelled substrate and demonstrate, if possible using radio-g.l.c., that the resulting individual hydrocarbons have similar specific activities.[3]

[1] G. Eglinton and R. J. Hamilton, 'Chemical Plant Taxonomy', ed. T. Swain, Academic Press, 1963, p. 187; A. G. Douglas and G. Eglinton, 'Comparative Phytochemistry', ed. T. Swain, Academic Press, 1966, p. 57.
[2] P. Gaskin, J. MacMillan, R. D. Firn, and R. J. Pryce, *Phytochemistry*, 1971, **10**, 1155.
[3] C. W. Bird, J. M. Lynch, and S. J. Pirt, *Chem. and Ind.* 1974, in the press; E. Merdinger and R. H. Frye, *J. Bacteriol.*, 1966, **91**, 1831.

For purposes of discussion the hydrocarbons produced by micro-organisms are conveniently sub-divided into methane, ethylene, other gaseous hydrocarbons, longer-chain hydrocarbons, and isoprenoids.

2 Methane

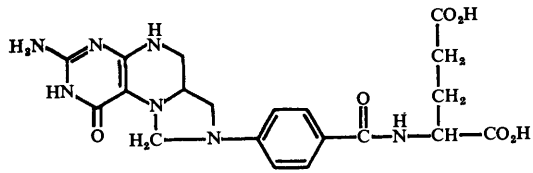
Methane is commonly encountered in nature wherever bacterial decomposition of organic material can occur under anaerobic conditions, as in swamps or the black muds of lakes. Some idea of the potential scale of methane formation is indicated by the fact that the anaerobic fermentation of sludge in modern sewage plants provides an ample supply of methane to generate all their power requirements. It is also produced in the digestive tracts of animals, to the extent of some 200 l per day in the case of a large cow, and a recent reviewer[4] has commented: 'if the legendary dragon ever existed it was probably a ruminant . . . however, the biochemistry of the ignition mechanism escapes modern-day biochemists'.

The small group of bacteria primarily responsible for methane formation have proved difficult to obtain in pure culture. Those currently known[4,5] as pure cultures are *Methanobacterium ruminantium*, *M. formicicum*, *M. mobilis*, *Methanosarcina barkeri*, *Methanococcus sp.*, *M. vannielii*, and a *Methanospirillum sp.*, which cover a variety of morphological types. The preferred substrates are generally hydrogen and carbon dioxide or formate. Exceptionally *Methanosarcina barkeri* will also grow on methanol, or acetate.[6] The problems which can be encountered in working in this area are illustrated by work with a culture described as *Methanobacillus omelianskii* which has played an important role in these studies. The culture produced methane in an ethanol–carbonate mineral medium, and the specific activity of the methane was the same as that of the added [^{14}C]carbon dioxide when the substrate was unlabelled ethanol.[7] Thus the ethanol was simply acting as a hydrogen source. Eventually it was found[8] that *Methanobacillus omelianski* was in fact a symbiotic association of two organisms, one of which, designated *Methanobacterium M.O.H.*, produced methane when grown on carbon dioxide and hydrogen. The other organism, 'S', oxidizes ethanol to acetate and hydrogen and its growth is suppressed by accumulation of hydrogen.

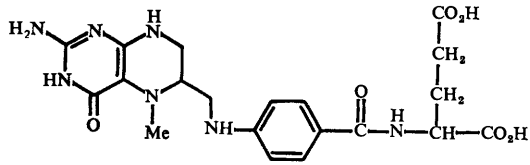
Little is known about the intermediate species between carbon dioxide and methane. Pyruvate, which was originally found to be active in the formation of methane by cell-free extracts,[9] has subsequently been shown to function as a source of carbon dioxide resulting from decarboxylation.[10] Formate functions

[4] R. S. Wolfe, *Adv. Microb. Physiol.*, 1971, **6**, 107.
[5] P H. Smith, *Developments Industrial Microbiol.*, 1966, **7**, 156.
[6] B. A. Blaylock and T. C. Stadtman, *Arch. Biochem. Biophys.*, 1966, **116**, 138.
[7] T. C. Stadtman and H. A. Barker, *Arch. Biochem.*, 1949, **21**, 256.
[8] M. P. Bryant, E. A. Wolin, M. J. Wolin, and R. S. Wolfe, *Archiv. Mikrobiol.*, 1967, **59**, 20.
[9] E. A. Wolin, M. J. Wolin, and R. S. Wolfe, *J. Biol. Chem.*, 1963, **238**, 2882.
[10] B. C. McBride, *cf.* ref. 4, p. 129.

in a similar fashion. It has been shown[11] that C-3 of serine can function as a methane precursor in *Methanobacterium M.O.H.* This carbon atom is transferred to tetrahydrofolate, forming *N*-5,*N*-10-methylenetetrahydrofolate (1), by the enzyme serine transhydroxymethylase. The species (1) is reduced to *N*-5-methyltetrahydrofolate (2) by an $NADH_2$-requiring reductase present in extracts



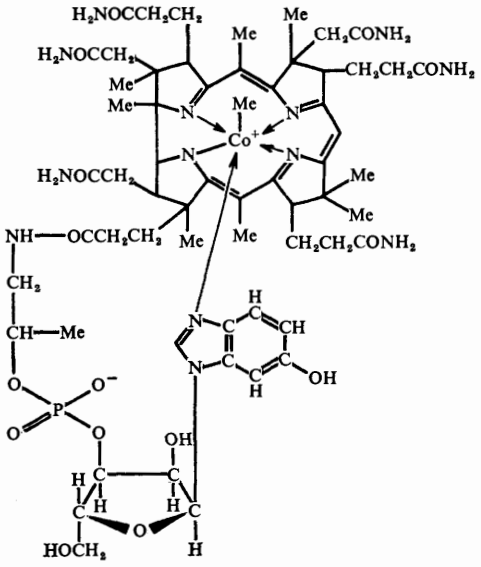
(1)



(2)

of the micro-organism. These extracts also readily generated [^{14}C]methane from *N*-5-$^{14}CH_3$-tetrahydrofolate. Presumably *N*-5-methyltetrahydrofolate provides the methyl group of methylcobalamin. Both methylcobalamin[12] and methyl-Co-5-hydroxybenzimidazolylcobamine (3),[13] the natural cobamide of *M. omelianski*, are excellent sources of methyl groups for methane formation in the presence of a cell extract, ATP, and a hydrogen atmosphere. Support for the supposition that methylcobalamins are normal intermediates in methane formation is provided by the observation that dichloromethane, chloroform, and carbon tetrachloride competitively inhibit methane formation both in rumen fluids and from methylcobalamin in cell-free extracts. The inhibition results from reaction of these compounds with B_{12s}, forming a series of chloromethylcobalamins.[14] The $^{14}CH_3$-cobaloximes (4) can also function as a source

[11] J. M. Wood, A. M. Allam, W. J. Brill, and R. S. Wolfe, *J. Biol. Chem.*, 1965, **240**, 4564.
[12] T. C Stadtman, *Ann. Rev. Microbiol.*, 1967, **21**, 121; M. J. Wolin, E. A. Wolin, and R. S. Wolfe, *Biochem. Biophys. Res. Comm.*, 1963, **12**, 464.
[13] J. M. Wood, M. J. Wolin, and R. S. Wolfe, *Biochemistry*, 1966, **5**, 2381.
[14] J. M. Wood, F. S. Kennedy, and R. S. Wolfe, *Biochemistry*, 1968, **7**, 1707.



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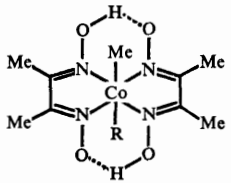


(4)

of methyl groups under these conditions.[15] The rate of methane formation is particularly affected by the nature of the axial ligand, L, the greatest activity being observed with easily displaced ones such as water and pyridine. Some methyl-group transfer to B_{12} has been observed with this system in the absence of ATP, but it is not known whether this occurs under the normal conditions of methane generation. Neither ethyl- nor n-propyl-cobaloxime produce ethane or propane under comparable conditions.

With *Methanosarcina barkeri*, for which methanol functions as the methyl donor and B_{12s} as the methyl acceptor, the necessary components of the methyl-

[15] B. C. McBride, J. M. Wood, J. W. Sibert, and G. N. Schrauzer, *J. Amer. Chem. Soc.*, 1968, **90**, 5276.

transfer reaction are ferredoxin, a corrinoid protein, an unidentified protein, an unknown heat-stable cofactor, ATP, magnesium ions, and a hydrogen atmosphere.[16]

So far as the subsequent formation of methane from methylcobalamin is concerned using cell-free extracts from *M. omelianski*, one mole of ATP is used for every mole of methane liberated.[17] In addition two protein fractions were required and a reduced flavin adenine dinucleotide-generating system.

Recently the synthesis of small amounts of methane by disrupted cells of *Desulphovibrio desulphuricans*, *Desulphotomaculum ruminis*, and *Clostridium pasteurianum* has been demonstrated.[18] The methyl group of pyruvate is the precursor of the methane and not the carboxy-group as in *Methanobacterium* extracts.[9] In one *Desulphovibrio sp.* methane formation was shown to involve B_{12}, coenzyme A, thiamine pyrophosphate, magnesium ions, and acetyl phosphate. Replacement of pyruvate by α-ketobutyrate led to formation of ethane.

3 Ethylene

The biological formation of ethylene has attracted increasing attention[19] since the accidental discovery in 1901 that it hastened the ripening of fruits.[20] It is now established as a plant-growth regulator and amongst the actions attributed to the gas are the breaking of dormancy, regulation of swelling and elongation, hypertrophy, promotion of adventitious roots, modification of root growth, promotion of root hairs, epinasty, hook closure, inhibition of leaf expansion, control of flower induction, exudation, ripening, senescence and abscission, inhibition of root nodulation, and induction of soil fungistasis. In animals, very much larger concentrations of ethylene (>80% in oxygen) are needed to produce readily observable physiological effects, primarily anaesthesia.[21]

Following earlier demonstrations that plants could produce ethylene, the first indications of microbial production were provided by the observation that the respiratory activity of citrus fruits was increased when they were infected by the green mould *Penicillium digitatum*.[22] It was subsequently shown that *P. digitatum* formed ethylene when grown in pure culture. Ethylene has been implicated in pathogenesis by a number of other micro-organisms (see Table). In the case of *Ceratocystis fimbriata* infection of sweet potato roots, the ethylene produced induces the formation of isocoumarins, which provide resistance to

[16] B. A. Blaylock, *Arch. Biochem. Biophys.*, 1968, **124**, 314.
[17] J. M. Wood and R. S. Wolfe, *J. Bacteriol.*, 1966, **92**, 696.
[18] J. R. Postgate, *J. Gen. Microbiol.*, 1969, **57**, 293.
[19] F. B. Abeles, 'Ethylene in Plant Biology', Academic Press, New York and London, 1973.
[20] D. Neljubov, *Beih. Bot. Zentralbl.*, 1901, **10**, 128.
[21] A. B. Luckhardt and J. B. Carter, *J. Amer. Med. Assoc.*, 1923, **80**, 1440; A. B. Luckhardt and D. Lewis, *ibid.*, 1923, **81**, 1851.
[22] J. B. Biale, *Science*, 1940, **91**, 458; J. B. Biale and A. D. Shepherd, *Amer. J. Bot.*, 1941, **28**, 263; E. V. Miller, J. R. Winston, and D. F. Fisher, *J. Agric. Res.*, 1940, **60**, 269.

further fungal attack.[24] Most pathogens, with the exception of *P. digitatum* and *P. solanacearum*,[32] produce little or no ethylene in pure culture so that the plant must be supplying the biosynthetically necessary substrates. The only human pathogens shown to produce ethylene are the dimorphic fungi *Blastomyces dermatitidis*, *B. braziliensis*, and *Histoplasma capsulatum*.[33] A recent survey found that 58 species of fungi out of 228 examined produced ethylene, *Aspergillus clavatus* being by far the most prolific.[34]

Ethylene has been observed in anaerobic soils at concentrations sufficient, under laboratory conditions, to affect the root extension of cereals.[35] The microbial origin of this ethylene was indicated by inhibition of its formation following sterilization by autoclaving or γ-irradiating the soil.[36] By enriching soil with methionine and glucose, which are substrates for ethylene formation,

Table *Ethylene production by some plant pathogens*

Pathogen	*Host*	*Symptom*	*Reference*
Penicillium digitatum	Citrus fruits	Fruit rot	22, 41—45, 61—64
Ceratocystis fimbriata	Sweet potato	Black rot	23, 24, 25
Erwinia carotovora *Xanthomonas campestris*	Cauliflower	Soft rot	26
Botrytis sp.	Carnation	Flower damage	27
Fusarium oxysporum	Tomatoes	Leaf wilt	28
	Tulip	Stunted growth, blasting of flower buds	29
Erysiphe graminis	Barley	Powdery mildew	30
Sclerotina fructigena	Apple	Brown rot	31
Pseudomonas solanacearum	Banana	Early ripening	32
	Tomato	Wilt	
	Tobacco	Wilt	

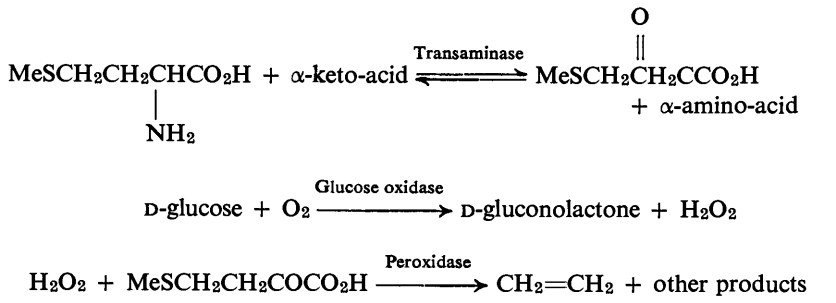
[23] E. Chalutz and J. E. DeVay, *Phytopathology*, 1969, **59**, 750; E. Chalutz, J. E. DeVay, and E. C. Maxie, *Plant Physiol.*, 1969, **44**, 235.
[24] S. Sakai, H. Imaseki, and I. Uritani, *Plant and Cell Physiol.*, 1970, **11**, 737.
[25] Y. Kato and I. Uritani, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 2601.
[26] B. M. Lund and L. W. Mapson, *Biochem. J.*, 1970, **119**, 251.
[27] W. H. Smith, O. F. Meigh, and J. C. Parker, *Nature*, 1964, **204**, 92.
[28] A. E. Dimond and P. E. Waggoner, *Phytopathology*, 1953, **43**, 663.
[29] W. J. de Munk and M. de Rooy, *Hort. Science*, 1971, **6**, 40; W. J. de Munk, *Netherlands J. Plant Pathol.*, 1973, **79**, 41.
[30] E. C. Hislop and M. A. Stahmann, *Physiol. Plant Pathol.*, 1971, **1**, 297.
[31] E. C. Hislop, G. V. Hoad, and S. A. Archer in 'Fungal Pathogenicity and the Plant's Response', ed. R. J. W. Bryde and C. V. Cutting, Academic Press, London, 1973, p. 87; E. C. Hislop, S. A. Archer, and G. V. Hoad, *Phytochemistry*, 1973, **12**, 2081.
[32] H. T. Freebairn and I. W. Buddenhagen, *Nature*, 1964, **202**, 313.
[33] W. J. Nickerson, *Arch. Biochem.*, 1948, **17**, 225.
[34] L. Ilag and R. W. Curtis, *Science*, 1968, **159**, 1357.
[35] K. A. Smith and R. S. Russell, *Nature*, 1969, **222**, 769; K. A. Smith and P. D. Robertson, *ibid.*, 1971, **234**, 148.
[36] K. A. Smith and S. W. F. Restall, *J. Soil Sci.*, 1971, **22**, 430.

the common soil fungus, *Mucor hiemalis*, and two yeasts, *Trichosporon cutaneum* and *Candida vartiovaari*, were isolated and shown to produce ethylene when grown in pure culture.[37] Subsequent work by others has confirmed the substrate requirements for ethylene formation by soil micro-organisms.[38] Considerable variability in ethylene production was encountered during attempts to study the physiology of its formation in shaken flasks, whereas consistency was obtained with chemostat cultures.[39] Higher yields of ethylene per gram of organism were obtained from the chemostat than from batch cultures and the amounts were further increased at lower growth rates.[40] The latter observation provides a possible explanation for the higher ethylene yields in chemostat cultures, where the specific growth rate is usually lower. More ethylene per gram of organism is formed as the concentration of dissolved oxygen is increased, but it seems that anaerobic conditions are necessary in the soil in order to release substrates from the soil organic matter for ethylene formation.[39]

Undoubtedly some of the foregoing observations with *M. hiemalis* must also be borne in mind when considering similar physiological studies with *Penicillium digitatum*. Here various workers[41–43] have shown that carbon sources such as serine, sugars, malate, α-alanine, and ethanol promote ethylene formation. There is marked disagreement as to whether ethylene production is higher in stationary[44] or in shaken[45] cultures. An additional complication is indicated by the wide variation in ethylene production observed[44] with single spore cultures. In stationary cultures at least the amount of ethylene produced is not proportional to growth of the organism, the maximum production occurring after completion of mycelial growth.[44]

As in many plants,[19] the precursor of ethylene formation by *E. carotovora*,[26] *P. solanacearum*,[46] and *M. hiemalis*[37,39] is methionine. In the latter case ethionine is equally acceptable but none of a wide range of other substrates including pyruvate and ethanol (see below). A pathway for the conversion of methionine into ethylene, established using cell-free systems extracted from cauliflower florets, is summarized in Scheme 1. The first stage is the conversion of methionine by a transaminase into 4-methylmercapto-2-oxobutyric acid (KMBA).[47] This compound is then converted into ethylene by a peroxidase enzyme in the presence of *p*-hydroxybenzoic acid and methanesulphinic acid.[48,49] The necessary hydrogen

[37] J. M. Lynch, *Nature*, 1972, **240**, 45.
[38] E. J. Dasilva, E. Henriksson, and L. E. Henriksson, *Plant Sci. Letters*, 1974, **2**, 63.
[39] J. M. Lynch and S. H. T. Harper, *J. Gen. Microbiol.*, 1974, **80**, 187.
[40] J. M. Lynch and S. H. T. Harper, unpublished observations.
[41] C. L. Fergus, *Mycologia*, 1954, **46**, 543.
[42] C. T. Phan, *Rev. générale Bot.*, 1962, **69**, 505.
[43] B. A. Sprayberry, W. C. Hall, and C. S. Miller, *Nature*, 1965, **208**, 1322.
[44] D. H. Spalding and M. Lieberman, *Plant Physiol.*, 1965, **40**, 645.
[45] M. Meheriuk and M. Spencer, *Canad. J. Bot.*, 1964, **42**, 337.
[46] B. T. Swanson, H. F. Wilkins, and B. Kennedy, *Hort. Science*, 1972, **7**, 26.
[47] L. W. Mapson, J. F. March, and D. A. Wardale, *Biochem. J.*, 1969, **115**, 653.
[48] L. W. Mapson and A. Mead, *Biochem. J.*, 1968, **108**, 875.
[49] L. W. Mapson, R. Self, and D. A. Wardale, *Biochem. J.*, 1969, **111**, 413.



Scheme 1

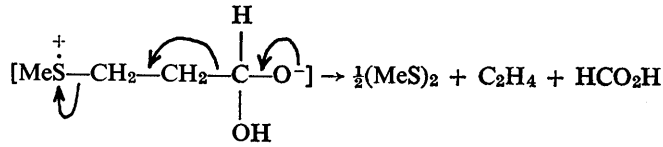
peroxide is generated by a glucose oxidase. Other workers have questioned[50,51] the role of KMBA in ethylene formation, since in some instances it is a far less satisfactory precursor than methionine. The role of the soft-rot bacterium *Erwinia carotovora* in promoting ethylene production in cauliflower florets appears to be the generation of pectic enzymes, which release and activate a glucose oxidase enzyme from the plant cell walls, thus increasing hydrogen peroxide production.[26] The bacterium does not produce ethylene in pure culture.

The filtered culture medium from *Mucor hiemalis* grown in shaken flasks has been found to contain a species which can generate ethylene without enzymic intervention.[52] The production of ethylene is stimulated by change of the normal pH of 6 to 1. This may be due to release of iron from the citrate chelate present in the medium since addition of ferrous iron greatly increases ethylene production. Ferric ions are rather less effective than ferrous ones, while cupric and manganous ones are without effect. Addition of ninhydrin, semicarbazide, or 2,4-dinitrophenylhydrazine to the filtered culture medium inhibits ethylene formation. So far the ethylene precursor has resisted positive identification.

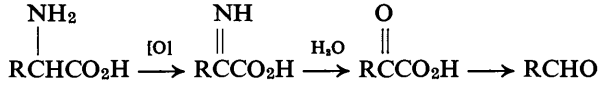
The foregoing observations draw attention to a basic difficulty in deciding to what extent ethylene production from methionine is enzyme-mediated. A variety of *in vitro* systems have been found which will effect this process including copper(II)–ascorbate,[54] peroxidase–manganese(II)–sulphite–phenol,[51,53] γ-irradiation,[55] and flavin mononucleotide[53] or flavin adenine dinucleotide[52] with light. In at least some instances it has been shown that methional or KMBA also yields ethylene. Additionally, the combination peroxidase, *p*-hydroxybenzoate, indolyl-3-acetic acid, and benzenesulphinate converts KMBA into ethylene.[56] It has been suggested that a free-radical mechanism[53] is responsible

[50] M. Lieberman and A. T. Kunishi, *Plant Physiol.*, 1971, **47**, 576.
[51] A. H. Baur, S. F. Young, H. K. Pratt, and J. B. Biale, *Plant Physiol.*, 1971, **47**, 696.
[52] J. M. Lynch, *J. Gen. Microbiol.*, in the press.
[53] S. F. Yang, *Arch. Biochem., Biophys.*, 1967, **122**, 481; *J. Biol. Chem.*, 1969, **244**, 4360.
[54] M. Lieberman, A. T. Kunishi, L. W. Mapson, and D. A. Wardale, *Biochem. J.*, 1965, **97**, 449.
[55] J. M. Lynch, unpublished observations.
[56] L. W. Mapson and D. A. Wardale, *Phytochemistry*, 1972, **11**, 1371.

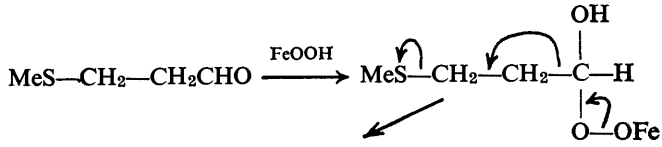
where a sulphinium ion is generated and breaks down to ethylene:



However, the failure to observe free radicals in the system by electron spin resonance studies appears to exclude this proposal. It seems more likely that the degradation of methionine follows the usual amino-acid oxidative route, although the mechanism of individual steps will vary according to the reagent:



The precise route from KMBA or methional to ethylene is a matter of conjecture. Experiments using the peroxidase–sulphite–manganese(II)–phenol system have shown[53] that whereas C-1 of methional is largely liberated as formic acid, C-2 of KMBA appears as carbon dioxide. The methylmercapto-group was detected as dimethyl disulphide, showing that prior oxidation of the sulphide group of methional or KMBA is not a prerequisite for ethylene formation. These observations are in keeping with the following speculative mechanism:



$$MeS^- + C_2H_4 + Fe{=}O + HCO_2H$$

In the case of KMBA the formic acid would be replaced by oxalic acid, which would probably be oxidized further to carbon dioxide. Experiments using both crude enzyme and model systems have shown that peptides containing *C*-terminal methionine residues are equally acceptable substrates for ethylene production.[57]

Apart from its formation from methionine, there is evidence that in plants and mammalian systems ethylene can also originate from oxidative breakdown of linolenate.[58] The extrapolation to a biological context is readily envisaged of the generation of ethylene from, *inter alia*, monoethyl phosphate and sulphate in the presence of ferrous sulphate and a peroxide[59] and the ethylene-forming

[57] H. S. Ku and A. C. Leopold, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 1155; D. M. Demorest and M. A. Stahmann, *Plant Physiol.*, 1971, **47**, 450.
[58] W. B. McGlasson, *Biochem. Fruits and Products*, 1970, **1**, 475.
[59] J. Kumamoto, H. Dollwet, and J. H. Lyons, *J. Amer. Chem. Soc.*, 1969, **91**, 1207.

breakdown of 2-chloroethanephosphonic acid in acid solution.[60] The need to bear in mind other routes which could lead to the biogenetic formation of ethylene is indicated by reports which throw doubt on the universality of the methionine route. For example tracer studies of *Ceratocystis fimbriata* infection of sweet potato tissue indicate that ethylene formation occurs not only by the methionine route, but also by an acetate route and by an as yet unidentified pathway.[25]

There is also disagreement as to whether methionine is a precursor of ethylene in *P. digitatum*.[61,62] One study concluded that uniformly labelled methionine was not converted into labelled ethylene,[61] whereas another found that it was rather poorly utilized but showed that C-3 and C-4 were specifically incorporated into ethylene.[62] Several groups[61–63] agree that acetate gives better incorporation and that C-2 but not C-1 is utilized. Incorporation of ^{14}C into ethylene was also found with serine (C-3),[43] succinic acid (C-2 and C-3), fumaric acid (C-2 and C-3), DL-malate (C-3), β-alanine (C-2), acrylate (C-2), propionate (C-3$\gg$ C-2),[62] and pyruvate (C-3).[63] Presumably these intermediates are incorporated *via* the Krebs cycle. Suggestions that ethylene might be formed by dehydration of ethanol are apparently excluded by the observation that C-2 is incorporated more than four times as efficiently as C-1.[63] The fact that all of the foregoing utilization studies use *P. digitatum* mycelial mats rather than cell-free systems raises the question as to whether the poor incorporation of methionine into ethylene could be due to transport problems. However, the production of ethylene was not affected[64] by rhizobitoxine[65] (5), which inhibits ethylene

$$HOCH_2CH(NH_2)CH_2OCH{=}{=}CHCHNH_2CO_2H$$

(5)

biosynthesis in sorghum seedlings and in senescent apple tissues.[64] Rhizobitoxine inhibits methionine biosynthesis by irreversibly inactivating the enzyme β-cystathionase both in bacteria[66] and plants[67].

Finally it must be emphasized that controls are always very important in ethylene studies as the gas can easily originate from non-biological sources such as rubber and plastic.[68]

[60] A. R. Cooke and D. I. Randall, *Nature*, 1968, **218**, 1974.
[61] D. L. Ketring, R. E. Young, and J. B. Biale, *Plant and Cell Physiol.*, 1968, **9**, 617.
[62] D. W. Jacobsen and C. H. Wang, *Plant Physiol.*, 1968, **43**, 1959.
[63] M. S. Gibson and R. E. Young, *Nature*, 1966, **210**, 529.
[64] L. D. Owens, M. Lieberman, and A. Kunishi, *Plant Physiol.*, 1971, **48**, 1.
[65] L. D. Owens, J. F. Thompson, R. G. Pitcher, and T. Williams, *J.C.S. Chem. Comm.*, 1972, 714.
[66] L. D. Owens, S. Guggenheim, and J. Hilton, *Biochim. Biophys. Acta*, 1968, **158**, 219.
[67] J. Giovanelli, L. D. Owens, and S. H. Mudd, *Biochim. Biophys. Acta*, 1971, **227**, 671.
[68] D. F. Meigh, *Nature*, 1962, **196**, 345; J. V. Jacobsen and W. B. McGlasson, *Plant Physiol.* 1970, **45**, 631; E. P. Kavanagh and J. R. Postgate, *Lab. Practice*, 1970, **19**, 159; D. W. Pritchard and A. F. Ross, *Plant Physiol.*, 1972, **49**, 564; B. Thake and P. R. Rawle, *Arch. Mikrobiol.*, 1972, **85**, 39.

4 Other Short-chain Hydrocarbons

Although gases such as ethane, propane, propylene, and higher hydrocarbons have been observed in soils[35,36] and may well be of microbial origin the matter has received no attention. However, the recent identification[69] of n-hexane as the gamone of the seaweed *Fucus vesiculosus* suggests that such unprepossessing molecules may have unsuspected physiological properties.

Probably one of the most exotic microbial hydrocarbons is hexa-1,3,5-triyne, the major volatile product from the Basidiomycete *Fomes annosus*.[70] This compound inhibits the growth of other fungi[71,72] and plants.[72,73] Subsequent screening of some 37 further species of the genus *Fomes* indicated that formation of hexatriyne was restricted to some strains of *F. annosus*.[73] Substantial amounts of methyl chloride were produced by some of the other *Fomes* species examined.

Acetylene is not produced in appreciable concentrations by micro-organisms or soils but it is used as a substrate to form ethylene in the now well-established test[74] for the nitrogen-fixing enzyme, nitrogenase.

5 Longer-chain Hydrocarbons

Longer-chain hydrocarbons have a role as potential microbial fossils[75] and the study of the microbial origin of these, together with the isoprenoid ones considered in the following section, has greatly accelerated since this was recognised. The rationale behind this proposition is that hydrocarbons are the most stable group of naturally occurring compounds and are likely to retain much of their original architecture over very long periods of time. As the oldest fossils are of bacteria and algae,[76] some more than three billion years old, there has been particular incentive to compare their chemical composition with those of modern micro-organisms.

Plant hydrocarbon fractions generally exhibit a marked predominance of alkanes possessing odd numbers of carbon atoms.[1] With certain exceptions, micro-organisms do not show this marked alternation in relative abundances between odd and even carbon number hydrocarbons. As far as alkanes are

[69] J. R. Hlubucek, J. Hora, T. P. Toube, and B. C. L. Weedon, *Tetrahedron Letters*, 1970, 5163.
[70] A. T. Glen, S. A. Hutchinson, and N. J. McCorkindale, *Tetrahedron Letters*, 1966, 4223.
[71] C. M. Dick and S. A. Hutchinson, *Nature*, 1966, **211**, 868.
[72] A. T. Glen and S. A. Hutchinson, *Trans. Brit. Mycol. Soc.*, 1973, **61**, 583.
[73] M. I. Cowan, A. T. Glen, S. A. Hutchinson, M. E. MacCartney, J. M. Mackintosh, and A. M. Moss, *Trans. Brit. Mycol. Soc.*, 1973, **60**, 347.
[74] J. R. Postgate, in 'The Chemistry and Biochemistry of Nitrogen Fixation', ed. J. R. Postgate, Plenum Press, London, 1971, p. 311.
[75] M. Calvin, 'Chemical Evolution', Oxford University Press, 1969.
[76] E. S. Barghoorn, *Scientific American*, 1971, **224**, 30.

concerned algae[77-92] generally have a range of *ca.* C_{16}—C_{33} with n-heptadecane as the major component. Fungi[88, 93-98] have a similar range of alkanes with nonacosane as the most abundant member, but the range can depend on the carbon source used for growth.[99] The yeasts[88,100,101] and bacteria[82,83,88,89,102-105] with similar ranges of alkanes often have *ca.* C_{18} and *ca.* C_{27} as the most abundant members. The qualitative and quantitative distribution of alkanes and alkenes in micro-organisms has been tabulated.[106] There are few reports of hydrocarbons below C_{14}; however, this may partly reflect on evaporation losses during extraction.

In the wheat fungal pathogens, *Tilletia sp.*, the spores have a similar alkane distribution to that of the uninfected wheat kernel, suggesting that the *Tilletia* alkanes are derived from the host,[94] whereas that of the spores of the smut *Ustilago maydis* is quite different from those observed for healthy or infected corn tissues.[95] The sclerotia of the fungus *Sclerotinia sclerotiorum*, as obtained from pea- and bean-cleaning operations, were found to contain a hydrocarbon in the C_{25}—C_{29} range in addition to its normal C_{13}—C_{19} range of n-alkanes.[107]

[77] R. C. Clark and M. Blumer, *Limnol. Oceanog.*, 1967, **12**, 79.
[78] S. W. G. Fehler and R. J. Light, *Biochemistry*, 1970, **9**, 418.
[79] E. Gelpi, J. Oró, H. J. Schneider, and E. O. Bennett, *Science*, 1968, **161**, 700.
[80] E. Gelpi, H. Schneider, J. Mann, and J. Oró, *Phytochemistry*, 1970, **9**, 603.
[81] J. Han, E. D. McCarthy, M. Calvin, and M. H. Benn, *J. Chem. Soc.* (*C*), 1968, 2785.
[82] J. Han, E. D. McCarthy, W. Van Hoeven, M. Calvin, and W. H. Bradley, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **59**, 29.
[83] J. Han and M. Calvin, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **64**, 436.
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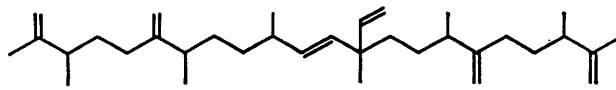
[85] J. Han and M. Calvin, *Chem. Comm.*, 1970, 1490.
[86] I. Iwata, H. Nakata, M. Mazushima, and Y. Sakurai, *Agric. and Biol. Chem.* (*Japan*), 1961, **25**, 319.
[87] I. Iwata and Y. Sakurai, *Agric. and Biol. Chem.* (*Japan*), 1963, **27**, 253.
[88] J. G. Jones, *J. Gen. Microbiol.*, 1969, **59**, 145.
[89] J. Oró, T. G. Tornabene, D. W. Nooner, and E. Gelpi, *J. Bacteriol.*, 1967, **93**, 1811.
[90] G. W. Patterson, *J. Phycol.*, 1967, **3**, 22.
[91] K. Stransky, M. Streibl, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1968, **33**, 416.
[92] K. Winters, P. L. Parker, and C. V. Baalen, *Science*, 1969, **163**, 467.
[93] C. W. Bird, J. M. Lynch, and S. J. Pirt, *Chem. and Ind.*, 1974, in the press.
[94] J. L. Laseter, W. M. Hess, J. D.Weete, D. L. Stocks, and D. J. Weber, *Canad. J. Microbiol.*, 1968, **14**, 1149.
[95] J. L. Laseter, J. Weete, and D. J. Weber, *Phytochemistry*, 1968, **7**, 1177; J. L. Laseter, J. Oró, and D. J. Weber, *Phytopathology*, 1966, **56**, 886.
[96] J. Oró, J. L. Laseter, and D. Weber, *Science*, 1966, **154**, 399.
[97] J. D. Weete, J. L. Laseter, D. J. Weber, W. M. Hess, and D. L. Stocks, *Phytopathology*, 1969, **59**, 545.
[98] D. J. Fisher, P. J. Holloway, and D. V. Richmond, *J. Gen. Microbiol.*, 1972, **72**, 71.
[99] J. D. Walker and J. J. Cooney, *Appl. Microbiol.*, 1973, **26**, 705.
[100] J. Baraud, C. Cassagne, L. Genevois, and M. Joneau, *Compt. rend.*, 1967, **265**, *D*, 83; M. Fabre-Joneau, J. Baraud, and C. Cassagne, *Compt. rend.*, 1969, **268**, *D*, 2282.
[101] E. Merdinger and E. M. Devine, *J. Bacteriol.*, 1965, **89**, 1488.
[102] C. W. Bird, J. M. Lynch, S. J. Pirt, W. W. Reid, C. J. W. Brooks, and B. S. Middleditch, *Nature*, 1971, **230**, 473.
[103] J. B. Davis, *Chem. Geol.*, 1968, **3**, 155.
[104] J. G. Jones and B. V. Young, *Arch. Mikrobiol.*, 1970, **70**, 82.
[105] G. Rebel, J. Barth, J. Viret, and P. Mandel, *Bull. Soc. Chim. biol.*, 1969, **51**, 1001.
[106] J. M. Lynch, Ph.D. Thesis, University of London, 1971.
[107] J. D. Weete, D. J. Weber, and D. Le Tourneau, *Arch. Mikrobiol.*, 1970, **75**, 59.

As this long-chain hydrocarbon was not formed during growth of the fungus on normal laboratory media the investigators suggested that it is specifically produced by a cell–fungal association; they do not seem to have considered the possibility that it is squalene derived from the plants.

Apart from *Tilletia foetida*, *T. caries*, and *T. controversa*, which contain small amounts of i-heptacosane, i-nonacosane, and i-hentriacontane,[94] i-alkanes have also been noted in *Sphacelothica reiliana*[96,97] (C_{25}, C_{27}, C_{29}, and C_{31}), *Ustilago maydis* (C_{27}, C_{29}, and C_{31}), and *Urocystis agropyii*[97] (C_{25}). By far the most prominent producers of branched-chain alkanes are the blue–green algae. In *Nostoc muscorum*,[81–85] *Anacystis cyanea*,[80] *Chroococcus turgidus*,[80] *Lyngbya aestuarii*,[80] and *Phormidium luridum*[81,83] the branched-chain alkanes are 7- and 8-methylheptadecanes, while in addition to a small amount of these *Chlorogloea fritschii*[81–83] contains a major amount of 4-methylheptadecane. Additionally *Chroococcus turgidus*[80] produces a small quantity of 6- and 7-methylhexadecanes.

Simple alkenes are also frequently encountered in yeast and algal hydrocarbons. In particular n-heptadec-1-ene is the principal hydrocarbon of *Chlorella pyrenoidosa*[80,108] and occurs in lesser amounts in *Anacystis nidulans* and *Scenedesmus quadricauda*.[80] The principal hydrocarbon of the latter organism is a heptacosene, and several alkenes (C_{23}, C_{25}, C_{26}, and C_{27}) are found in *Anacystis montana*.[79,80] The formation of pentacos-1-ene and heptacos-1-ene by *Chlorella vulgaris* under heterotrophic conditions in addition to the C_{17}—C_{36} alkanes formed autotrophically[90] emphasizes the potential role of growth conditions in determining the composition of hydrocarbon fractions. A more complex situation is presented by the widely distributed fresh-water alga *Botryococcus braunii*, of which three distinct physiological states are known.[109] Very little hydrocarbon synthesis occurs in large green cells, but in green active-state colonies the hydrocarbon fraction is *ca.* 20% of the dry weight, the principal components being *cis*-heptacosa-1,18-diene, *cis*-nonacosa-1,20-diene, and *cis*-hentriaconta-1,22-diene.[109,110] In the brown resting state, where hydrocarbons account for some 70% of dry weight, the predominant species are botryococcene and isobotryococcene in 9:1 ratio.[109,111] The structure deduced[112] for botryococcene (6) indicates that it is best regarded as a tetramethylated acyclic triterpene.



(6)

[108] C. W. Bird and R. A. Khan, unpublished observations.
[109] A. C. Brown, B. A. Knights, and E. Conway, *Phytochemistry*, 1969, **8**, 543.
[110] B. A. Knights, A. C. Brown, E. Conway, and B. S. Middleditch, *Phytochemistry*, 1970, **9**, 1317.
[111] J. R. Maxwell, A. G. Douglas, G. Eglinton, and A. McCormick, *Phytochemistry*, 1968, 7, 2157.
[112] R. E. Cox, A. L. Burlingame, D. M. Wilson, G. Eglinton, and J. R. Maxwell, *J.C.S. Chem. Comm.*, 1973, 284.

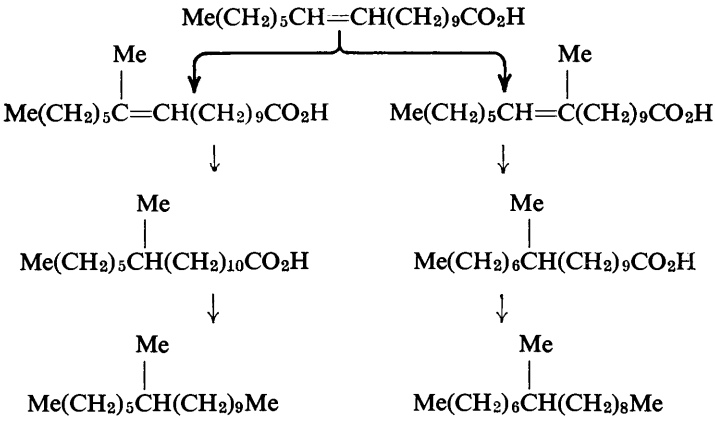
Considerable attention has been paid to the olefinic hydrocarbons of *Sarcina lutea* and other members of the family *Micrococcacae*.[113–124] Apart from the variations between different investigators who have used the same strain, considerable confusion arose because the micro-organism now provided by the American Type Culture Collection as ATCC 533 is not *S. lutea* and not the same as that used by the earlier workers.[113] The original strain is now referred to as FD 533.[117] In the case of this bacterium the alkenes account for over 90% of the hydrocarbon fraction with the C_{27}, C_{28}, and C_{29} families predominating. The C_{29} alkene family is comprised of *cis*-dimethylheptacos-13-enes having di-iso, iso–anteiso and di-anteiso terminations.[124] Similar iso and anteiso arrangements are present in the C_{27} and C_{28} alkenes. The position of the double bond in the C_{28} hydrocarbons is either Δ^{11}, Δ^{12}, or Δ^{13}, while for the C_{27} ones it is Δ^{11} or Δ^{12}. In ATCC 533 grown on the same medium the predominant families are C_{25}, C_{26}, and C_{27}, while for *S. lutea* ATCC 382, *S. flava*, and *S. subflava* the major alkenes are C_{27} and C_{29}.[122] The C_{29} alkenes are predominant in other *S. lutea* and *Micrococcus lysodeikticus* strains. It has been noted that the relative proportions of the members of each alkene family vary depending on whether a chemically defined medium, trypticase soy broth or nutrient broth, is employed.[115] Presumably this reflects the relative availabilities of isoleucine and valine in the growth media (see below). It has also been noted that, whereas in the early stationary phase only *ca.* 10% of the hydrocarbons were saturated, this proportion rises to 89% in the late stationary phase.[113,117]

Despite the aforementioned isolated observations that growth conditions can have marked effects on microbial hydrocarbon production, no systematic study has been reported. The authors' attempts to study such aspects of alkane production by *Aspergillus nidulans* were thwarted by the discovery that alkane synthesis and dissimilation occurred concurrently.[93] It seems likely that this could be a general phenomenon.

An overall picture is beginning to emerge of the biosynthetic pathways leading to hydrocarbon formation. Although much of the initial work was carried out on plants such as *Brassica oleracea* and *Nicotiana tabacum*,[125] some of the most informative results have been obtained with micro-organisms. Two pathways have been widely discussed. One of these, termed the elongation–decarboxylation

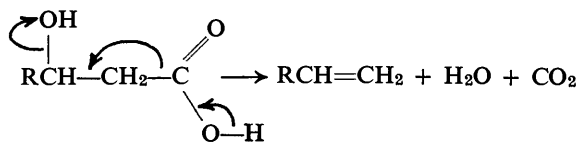
[113] P. W. Albro and C. K. Huston, *J. Bacteriol.*, 1964, **88**, 981.
[114] T. G. Tornabene, E. Gelpi, and J. Oró, *J. Bacteriol.*, 1967, **94**, 333.
[115] T. G. Tornabene, E. O. Bennett, and J. Oró, *J. Bacteriol.*, 1967, **94**, 344.
[116] T. G. Tornabene and J. Oró, *J. Bacteriol.*, 1967, **94**, 349.
[117] P. W. Albro and J. C. Dittmer, *Biochemistry*, 1969, **8**, 394.
[118] P. W. Albro and J. C. Dittmer, *Biochemistry*, 1969, **8**, 953.
[119] P. W. Albro and J. C. Dittmer, *Biochemistry*, 1969, **8**, 1913.
[120] P. W. Albro and J. C. Dittmer, *Biochemistry*, 1969, **8**, 3317.
[121] P. W. Albro, T. D. Meehan, and J. C. Dittmer, *Biochemistry*, 1970, **9**, 1893.
[122] T. G. Tornabene, S. J.Morrison, and W. E. Kloos, *Lipids*, 1970, **5**, 929; T. G. Tornabene and S. P. Markey, *ibid.*, 1971, **6**, 190.
[123] P. W. Albro, *J. Bacteriol.*, 1971, **108**, 213.
[124] P. W. Albro and J. C. Dittmer, *Lipids*, 1970, **5**, 320.
[125] P. E. Kolattukudy, *Lipids*, 1970, **5**, 259.

pathway, envisages the biosynthesis of long-chain fatty acids (*ca.* C_{16}) which are elongated in C_2 units and subsequently decarboxylated. Until recently this pathway appeared to be strongly supported by labelling experiments but there now appears to be an alternative explanation. A principal objection to the pathway was the general failure to observe the long-chain ($> C_{20}$) fatty acids which were postulated intermediates. However, it does seem to be responsible for the formation of the shorter-chain hydrocarbons, as isotopic labelling studies indicate[126] that palmitic acid and stearic acid are the respective precursors of the n-pentadecane and n-heptadecane of *Nostoc muscorum*. The accompanying 1:1 mixture of 7- and 8-methylheptadecanes is derived from *cis*-vaccenic acid (octadec-11-enoic acid) by addition of a methionine methyl group to the double bond followed by reduction and decarboxylation (Scheme 2).[126,127] In the case



Scheme 2

of *Anabaena variabilis* it has been established that the methyl group of [*Me*-$^2H_3$]methionine is incorporated intact, thereby excluding the intermediary formation of 11,12-methylenestearic acid.[127] Nothing is known about the decarboxylation step. In view of the availability of a double-bond-reducing system in these algae and the co-occurrence of heptadec-1-ene with this system, it is tempting to suggest that the decarboxylation step involves the concerted fragmentation of a β-hydroxy-acid,



[126] J. Han, H. W.-S. Chan, and M. Calvin, *J. Amer. Chem. Soc.*, 1969, **91**, 5156.
[127] S. W. G. Fehler and R. J. Light, *Biochemistry*, 1970, **9**, 418.

or a suitable derivative such as a phosphate ester. However, it should be noted that several micro-organisms are able to introduce a terminal[128] or internal[129] double-bond into an alkane substrate.

The other widely considered pathway for alkane biosynthesis commences with a Claisen-like condensation of two fatty acids, or suitable derivatives, followed by decarboxylation to the ketone and subsequent reduction of the carbonyl group:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\quad CO_2H \\ \qquad\qquad\qquad\qquad\quad | \\ R^1CH_2CO_2H + R^2CH_2CO_2H \longrightarrow R^1CHCOCH_2R^2 \\ \qquad\qquad\qquad\qquad\quad \downarrow -CO_2 \\ R^1CH_2CH_2CH_2R^2 \longleftarrow R^1CH_2COCH_2R^2 \end{array}$$

It will be noted that alkanes with an even number of carbon atoms in their chains can only result from coupling of fatty acids containing an even and an odd number of carbon atoms. While the relative rarity of odd carbon number fatty acids explains the predominance of odd carbon number alkanes in plants, no satisfactory explanation has been offered as to why the more equal distribution of odd and even carbon number alkanes occurs in micro-organisms, whose fatty-acid distributions are very similar to those of plants. The same strictures of course apply equally to the elongation–decarboxylation pathway.

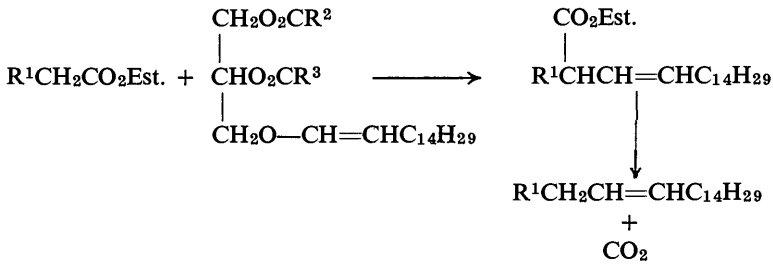
Convincing evidence demonstrates that the long-branched-chain alkenes of *S. lutea* are formed by a type of head-to-head condensation. Initial labelling studies showed that the anteiso groupings were derived from isoleucine and the iso groups from valine.[118] It is noteworthy that neither the resulting anteiso nor the iso fatty acids were incorporated into the alkenes to a degree proportional to their concentration in the total lipids. This has been interpreted as indicating that either a specific pool of fatty acids, possibly determined by the fatty-acid composition of a particular class of lipids, participates in the biosynthesis, or less likely that the enzymes involved have specificity for certain fatty acids. Experiments in which palmitate was added to *S. lutea* in the presence of substantial amounts of acetate resulted in its incorporation into hydrocarbons without any loss of its carboxy carbon.[119] The double bond in the resulting hydrocarbon is between C-1 and C-2 of the incorporated palmitic acid moiety. The decarboxylated fatty acid is derived from the lipid:

$$C_{14}H_{29}CH_2CO_2H + \mathit{RCH_2CO_2H} \longrightarrow C_{14}H_{29}CH{=}CH\mathit{CH_2R} + \mathit{CO_2}$$

Work with cell-free preparations showed that palmitoyl coenzyme A was incorporated faster into the hydrocarbons than was the free acid in the absence

[128] F. Wagner, W. Frahn, and U. Buhring, *Angew. Chem. Internat. Edn.*, 1967, **6**, 359; H. Iizuka, M. Iida, and S. Fujita, *Z. allg. Mikrobiol.*, 1969, **9**, 223.
[129] B. J. Abbott and L. E. Casida, *J. Bacteriol.*, 1968, **96**, 925.

of coenzyme A, but little incorporation of the carboxyl carbon occurred.[120] Further studies showed that the acyl moiety of triglycerides, fatty-acid methyl esters, and wax esters is incorporated only with decarboxylation.[121] Additionally it was found that palmitaldehyde was incorporated into hydrocarbons in preference to palmitic acid. Thus the long-chain ketones and alcohols which would result from head-to-head condensation of two molecules of fatty acid, and are present in *S. lutea*, would not be expected to be intermediates in hydrocarbon biosynthesis.[121] This expectation is confirmed by failure to observe their incorporation into the corresponding olefins. A future paper is due to appear[121,124] showing that the alk-1-enyl aliphatic group of a neutral plasmalogen is normally incorporated into the hydrocarbon rather than the aldehyde. The process now envisaged can be summarized in Scheme 3. As mentioned earlier this work has necessitated a reappraisal[125] of the evidence cited in support of the elongation–decarboxylation pathway for the formation of nonacosane in young pea and spinach leaves where the incorporation of [1-^{14}C]palmitic acid without loss of ^{14}C appeared to exclude the head-to-head condensation pathway, which in its original form required the loss of half the activity.



Scheme 3

The roles, if any, of these hydrocarbons in micro-organisms is uncertain. Fungi which are airborne have spore wall surfaces which are difficult to wet, and hydrocarbons probably contribute to this property.[98] This is important in plant disease because it means that the spores are more resistant to desiccation and to fungicidal sprays. It may be conjectured that hydrocarbons also play a part in the microbial cell wall. In this respect it has been shown[100] that in *Candida utilis* the alkanes and alkenes are located largely in the cell wall and cell contents.

6 Isoprenoid Hydrocarbons

Apart from the tetraterpenoid carotenoids[130] and botryococcene (see above) several triterpene hydrocarbons have been found in micro-organisms. The detection in yeasts and other fungi of squalene where it is clearly a sterol precursor

[130] B. C. L. Weedon in 'Carotenoids', ed. O. Isler, Birkhäuser Verlag, Basel, 1971, p. 29.

is not particularly surprising except that its reported occurrences[93,100,131–133] are far fewer than might have been expected. This may be due in part to analytical problems since confusion with n-alkanes can easily occur during g.l.c. analysis unless the hydrocarbon fraction is separated into straight-chain and branched-chain fractions by molecular sieving. Another factor may be the choice of growth conditions. It has been shown for example that *Saccharomyces cerevisiae* accumulates substantially more squalene when grown anaerobically than aerobically.[100,133] Another interesting observation is that in *Candida utilis* the squalene is located solely in the cytoplasmic membrane whereas the accompanying alkanes and alkenes are mainly in the cell walls and contents.[100]

Until very recently it was generally believed that prokaryotic organisms, the bacteria and blue–green algae, were incapable of synthesizing sterols and by implication squalene, in contradistinction to eukaryotic organisms. This is compatible with the important role of steroids in membrane formation and the apparent absence of internal membranes in prokaryotes. The recent observations of sterols in various blue–green algae[134,135] and bacteria[102,132,136] most of which are known to have extensive internal membrane systems, has been accompanied by the detection of squalene *per se* in these prokaryotes.[83,102,132,134,137,138] Particularly noteworthy is the methane-utilizing bacterium, *Methylococcus capsulatas*,[102] in which squalene accounts for 0.55% of the microbial dry weight! In *Staphylococcus aureus* squalene is accompanied by *cis*-12,13-dehydrosqualene but no sterol formation has been detected.[137] The extremely halophilic bacterium *Halobacterium cutirubrum* produces not only dehydrosqualene and squalene but also 2,3-dihydro- and 2,3,22,23-tetrahydro-squalene.[138] The proportion of reduced squalenes increases with age of the culture, suggesting that they result from stepwise terminal reduction.

Even more unexpected was the discovery[139] of the pentacyclic triterpene hop-22(29)-ene (7) in *M. capsulatus* and its recognition as the previously unidentified[80] hydrocarbon in several blue–green algae. Concurrently[140] it was found in the thermophilic bacterium *Bacillus acidocaldarius*, accompanied by minor amounts of hop-17(21)-ene (8), hopane, and possibly C_{31} homologues.

[131] K. J. Stone and F. W. Hemming, *Biochem. J.*, 1965, **96**, 14C; W. W. Epstein and G. V. Lear, *J. Org. Chem.*, 1966, **31**, 3434; H. P. Kaufmann, A. K. S. Ahmad, and S. S. Radwan, *Fette, Seifen, Anstrichm.*, 1966, **68**, 1010.

[132] K. Schubert, G. Rose, H. Wachtel, C. Horhold, and N. Ikekawa, *European J. Biochem.*, 1968, **5**, 246.

[133] D. Jollow, G. M. Kellerman, and A. W. Linnane, *J. Cell. Biol.*, 1968, **37**, 221.

[134] N. J. de Souza and W. R. Nes, *Science*, 1968, **162**, 363.

[135] R. C. Reitz and J. G. Hamilton, *Comp. Biochem., Physiol.*, 1968, **25**, 401.

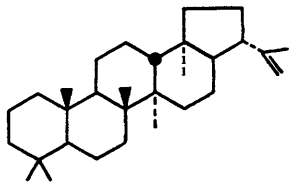
[136] K. Schubert, G. Rose, and C. Horhold, *Biochim. Biophys. Acta*, 1967, **137**, 168.

[137] G. Suzue, K. Tsukada, C. Nakai, and S. Tanaka, *Arch. Biochem. Biophys.*, 1968, **123**, 644; G. Suzue, K. Tsukada, and S. Tanaka, *Biochim. Biophys. Acta*, 1968, **164**, 88.

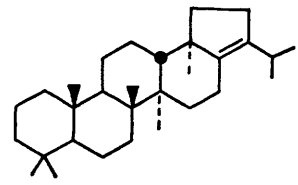
[138] T. G. Tornabene, M. Kates, E. Gelpi, and J. Oró, *J. Lipid Res.*, 1969, **10**, 294; S. C. Kushwaha, E. L. Pugh, J. K. G. Kramer, and M. Kates, *Biochim. Biophys. Acta*, 1972, **260**, 492; J. K. G. Kramer, S. C. Kushwaha, and M. Kates, *ibid.*, 1972, **270**, 103.

[139] C. W. Bird, J. M. Lynch, S. J. Pirt, and W. W. Reid, *Tetrahedon Letters*, 1971, 3189.

[140] M. de Rosa, A. Gambarcorta, L. Minale, and J. D. Bu'Lock, *Chem. Comm.*, 1971, 619; *Phytochemistry*, 1973, **12**, 1117.

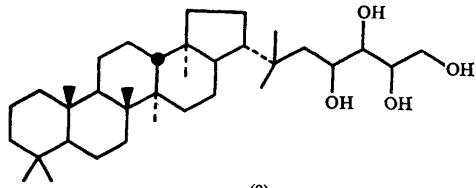


(7)



(8)

From a biosynthetic viewpoint these hopenes are almost certainly derived by acid-catalysed cyclization of squalene[141] and suggest the operation of a simpler process, as might have been anticipated on evolutionary considerations, than the squalene oxide cyclization which is generally responsible for steroid and triterpene synthesis. It should be noted in this context that *M. capsulatus* is the only one of these hopene-forming bacteria known to form sterols. The first indication that the hopenes may fulfil important physiological roles was provided by the isolation[142] of the compound (9) and a monounsaturated analogue, as well as 22-hydroxyhopane, from *Acetobacter xylinum.* These compounds were found specifically to promote the alignment of the extracellular cellulose microfibrils produced by this bacterium.[143]



(9)

7 Geochemical Aspects

Until fairly recently it was widely assumed that the organic compounds of sediments and oils were derived from plants and other higher organisms especially because prokaryotic organisms were believed incapable of producing precursors of the petroleum steranes and triterpanes. As more detailed knowledge has accrued of the chemical composition of the deposits on the one hand and of micro-organisms on the other, it has become apparent that there

[141] D. H. R. Barton, A. F. Gosden, G. Mellows, and D. A. Widdowson, *Chem. Comm.*, 1969, 184.
[142] H. J. Förster, K. Biemann, W. G. Haigh, N. H. Tattrie, and J. R. Colvin, *Biochem. J.*, 1973, **135**, 133.
[143] W. G. Haigh, H. J. Förster, K. Biemann, N. H. Tattrie, and J. R. Colvin, *Biochem. J.*, 1973, **135**, 145.

has been a substantial microbial contribution in many instances. Two recent reviews[144] on organic geochemistry render a detailed review of the subject in this article unnecessary and only particularly apposite examples will be mentioned.

Brief reference[145] to the role of *Botryococcus braunii* in the formation of boghead coals has already been made. It has also been concluded[146] that this alga is responsible for the oil content *inter alia* of certain Palaeozoic oil-bearing rocks. An illustration of the potential value of hydrocarbons as chemical fossils is provided by the observation[83,147] that the Pre-Cambrian ($>2700 \times 10^6$ years) Soudan Shale appears to contain the mixture of 7- and 8-methylheptadecanes that is a unique feature of the hydrocarbons of several blue–green algae. As the blue–green alga *Nostoc* is one of the types of microfossil believed to be present in some other ancient rocks it seems likely that an organism of this type contributed to the organic contents of the Soudan Shale.

Recently the Eocene Messel oil shale (50×10^6 years), which formed in shallow lakes, has been found to contain both hopane and homohopane.[148] Hopane is also one of the main triterpanes of the Green River Shale (60×10^6 years)[149] and triterpanes of the hopane type are widely present in petroleum[150] and a wide range of other sediments.[151] Until recently the hopane triterpenes had only been found in primitive plants, *i.e.* ferns, mosses, and lichens,[152] but their recent detection in blue–green algae and other bacteria (see above) suggests[139] that an appreciable proportion of the triterpanes and other components found in sediments and oil could be of bacterial origin.

[144] J. R. Maxwell, C. T. Pillinger, and G. Eglinton, *Quart. Rev.*, 1971, **25**, 571; P. Albrecht and G. Ourisson, *Angew. Chem. Internat. Edn.*, 1971, **10**, 209.
[145] K. B. Blackburn and B. N. Temperley, *Trans. Roy. Soc. Edinburgh*, 1936, **58**, 841.
[146] A. Traverse, *Micropaleontology*, 1944, **1**, 343.
[147] M. Calvin, 'Chemical Evolution', Oxford University Press, 1969, p. 82.
[148] A. Ensminger, P. Albrecht, G. Ourisson, B. J. Kimble, J. R. Maxwell, and G. Eglinton, *Tetrahedron Letters*, 1972, 3861.
[149] W. Henderson, V. Wollrab, and G. Eglinton, 'Advances in Organic Geochemistry', ed. P. A. Schenk and I. Havenaar, Pergamon Press, Oxford, 1968, p. 181.
[150] E. V. Whitehead, *Chem. and Ind.*, 1971, 1116.
[151] A. van Dorsselaer, A. Ensminger, C. Spyckerelle, M. Dastillung, O. Sieskind, P. Arpino, P. Albrecht, G. Ourisson, P. W. Brooks, S. J. Gaskell, B. J. Kimble, R. P. Philp, J. R. Maxwell, and G. Eglinton, *Tetrahedron Letters*, 1974, 1349.
[152] G. Berti and F. Bottari, 'Progress in Phytochemistry', ed. L. Reinhold and Y. Liwschitz, Wiley, London, 1968, p. 589.