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Life Sciences Division

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Melvin Calvin: Fuels from Plants

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This report offers a brief history of Prof. Melvin Calvin's (Figure 1) investigation into the potential use of plants and plant products as an economical source of hydrocarbon-like fuels. This work was carried out over a twenty year period, from 1968 until 1988, and involved the efforts of numerous students, co-workers and collaborators from around the world.

I. Fuel Oil from Plants

Dr. Calvin's initial interest in plants as a source of fuels arose from his interest in the origins of geologic hydrocarbons [McCarthy and Calvin 1967, Han et. al 1968] and from three concerns: 1) the need for renewable hydrocarbon based fuels that would not alter atmospheric CO_2 levels; 2) the finite pools of fossil hydrocarbon available; and 3) the need for increased domestic production of fuels and chemical feed stocks [Calvin 1975ab, 1977c, 1978g, 1979ab, 1984a, 1987ab].

Global warming, resulting from the so-called greenhouse effect, was an early concern of Calvin. The observed 8% increase in atmospheric carbon dioxide, 7 cm rise in mean sea level and reported increase in global temperatures over the past 100 years have been associated by many researchers with increased use of fossil hydrocarbon fuels. Fuels generated directly from plants would not have this effect, as any CO_2 generated in their combustion would have recently been fixed and reduced by the plant's photosynthetic process.

Figure 1. Melvin Calvin with an *Euphorbia lathyris* plant.

Calvin's second worry involved the growing awareness that the available stores of fossil fuels are being used up, and that the cost of extracting them, both monetarily and in energy, was increasing. Calvin feared that these facts would force a worldwide increase in the use of coal resources, which would exacerbate the global CO_2 problem.

Calvin's third concern was political, and derived from the geopolitical changes taking place in the early 1970s, when the Arab nations were nationalizing their oil stocks. A mid-eastern war and world politics resulted in an oil embargo, greatly affecting fuel availability in the United States. The local outcome of these global events was that Calvin had to wait in long lines for gasoline, resulting in a high level of personal frustration and an increased incentive to find alternative sources of fuel that could be generated from within the United States.

Calvin's overall goal was the identification and cultivation of selected species of green plants that could be harvested as a renewable fuel source on an "energy" farm. The product would need to have a high reduced-carbon (energy-rich) content that could easily be extracted from the total biomass, and the characteristics of the product must allow it to be used directly or be easily converted into high quality liquid fuel. In addition, the best plant candidates would be either adaptable to arid, non-agricultural lands, or be rain forest-based to provide an economically and environmentally safe use of tropical land. So the initial goal of the research was the identification of potential candidate species that met these criteria.

Calvin collected a variety of samples of herbaceous- and tree-derived hydrocarbons for study through travel and via contact with other scientists, but his main interests evolved around two plant species: the *Copaifera multijuga* tree from the Amazon region of Brazil, and the herbaceous *Euphorbia lathyris*, found in the arid grasslands of California.

The potential use of *Copaifera* for fuel stocks was discovered by Calvin on a trip to the Amazon region in 1978. The oil is collected by tapping the tree trunk twice a year (Figure 2). About 50 liters of oil can be collected per tree per year [Calvin 1980, 1984a]. The major components of this oil are the cyclic sesquiterpenes copaene, caryophyllene and bergamotene. These have the consistency of diesel oil, and as was demonstrated for Calvin in Brazil, can be used directly in a diesel engine. Preliminary studies on *Copaifera* sesquiterpene biosynthesis were performed by Cynthia Skrukrud in Calvin's laboratory as part of her thesis project [Skrukrud thesis 1987].

The majority of plant-derived fuel research performed in Calvin's laboratory was done with the *E. lathyris* plant. *E. lathyris* is a latex-bearing plant that grows wild in

California. As *Euphorbiaceae* latex commonly is enriched in reduced isoprenoids, hydrocarbon-like molecules derived in a manner similar to rubber, Calvin and Drs. John Otvos and Esther Nemethy initiated a program to study the potential of *E. lathyris* as an energy crop.

With initial funding from the Office of the Vice President for Agriculture, University of California, and under the leadership of Calvin, test plots of the plant were established first in 1977 at the U.C. South Coast Field Station in Santa Ana, CA and later at U.C. Davis to determine the agronomic conditions that maximize growth and yield (Figure 3). Results indicated that yields of 22 dry metric tons per hectare per year could be expected [Nemethy et. al LBL Report 1978]. The field-dried biomass from these plants can be fractionated by solvent extraction into three components: a heptane extractable, containing the hydrocarbons; a methanol extractable, containing the sugars, and the residue or bagasse, containing the ligno-cellulose material. Yields of 5% hydrocarbon, 30% sugar, and 65% residue are typical [Nemethy et. al 1981]. The energy return from the hydrocarbon fraction, expressed as the equivalent value in barrels of oil, was estimated in an SRI International report to be in the range of 4-10 barrels of oil per acre per year.

Figure 2. Tapping sap from a *Copaifera* tree in Brazil.

Figure 3. Keith Redenbaugh in a field of *Euphorbia lathyris* plants in Davis, CA.

To increase yields, Calvin undertook a program to understand the mechanisms of regulation of isoprenoid synthesis in *E. lathyris*. This involved an analysis of the structure of the latex isoprenoid components, an investigation into the path of their synthesis, a determination of the rate-limiting steps along the pathway, and an understanding of the subcellular organization of these steps and their association with numerous particles and organelles found in latex.

II. The Biochemistry and Physiology of *Euphorbia Lathyris*

Once *E. lathyris* was chosen as the target for extensive study, the first experimental priority was determining the structures of the isoprenoids in latex. The major components were found to be cyclic 30- and 31-carbon triterpenoids, possessing the 4-ring cyclic structure common to sterols. Early work by Drs. Hiroyuki Nishimura, Peter Neilsen and Nemethy and further work by Douglas Hawkins as part of his thesis project identified six major triterpenoid components in latex: euphol, lanosterol, cycloartenol, 24-methylenecycloartenol, 24-methylenelanosterol, and butyrospermol (Figure 4) [Neilsen et. al 1979; Nemethy et. al 1979; Hawkins thesis 1987]. In addition, 20-carbon diterpenes and their corresponding esters were found to be the latex. These were determined to promote cellular division [Bissell et. al 1981], and are thus thought to be responsible for the adverse effects associated with contact with *E. lathyris* latex, namely skin and mucous membrane irritation.

Once the hydrocarbon chemical structures were determined, the next step was to understand the biosynthetic mechanisms and subcellular structures responsible for their production. The first experiments, carried out by Nemethy, Dr. George Piazza and Skrukrud, examined the biosynthetic pathways of terpenoid biosynthesis. They performed *in vitro* assays with isolated latex and radiolabeled substrates. From these studies, they determined that the synthetic route was from acetate through the isoprenoid pathway, forming the cyclic 30-carbon triterpenol compounds and their corresponding triterpenol esters.

Though Nemethy and her co-workers found that acetate, pyruvate and mevalonate were utilized for triterpenoid biosynthesis, other intermediates of the isoprenoid pathway (i.e.. acetyl-CoA, hydroxymethylglutaryl-CoA, hydroxymethylglutarate or isopentenyl pyrophosphate) were not effective

precursors [Nemethy et. al 1983]. They concluded that there must be some subcellular organization or enzyme complexing that prevents access of many substrates for entry into the pathway.

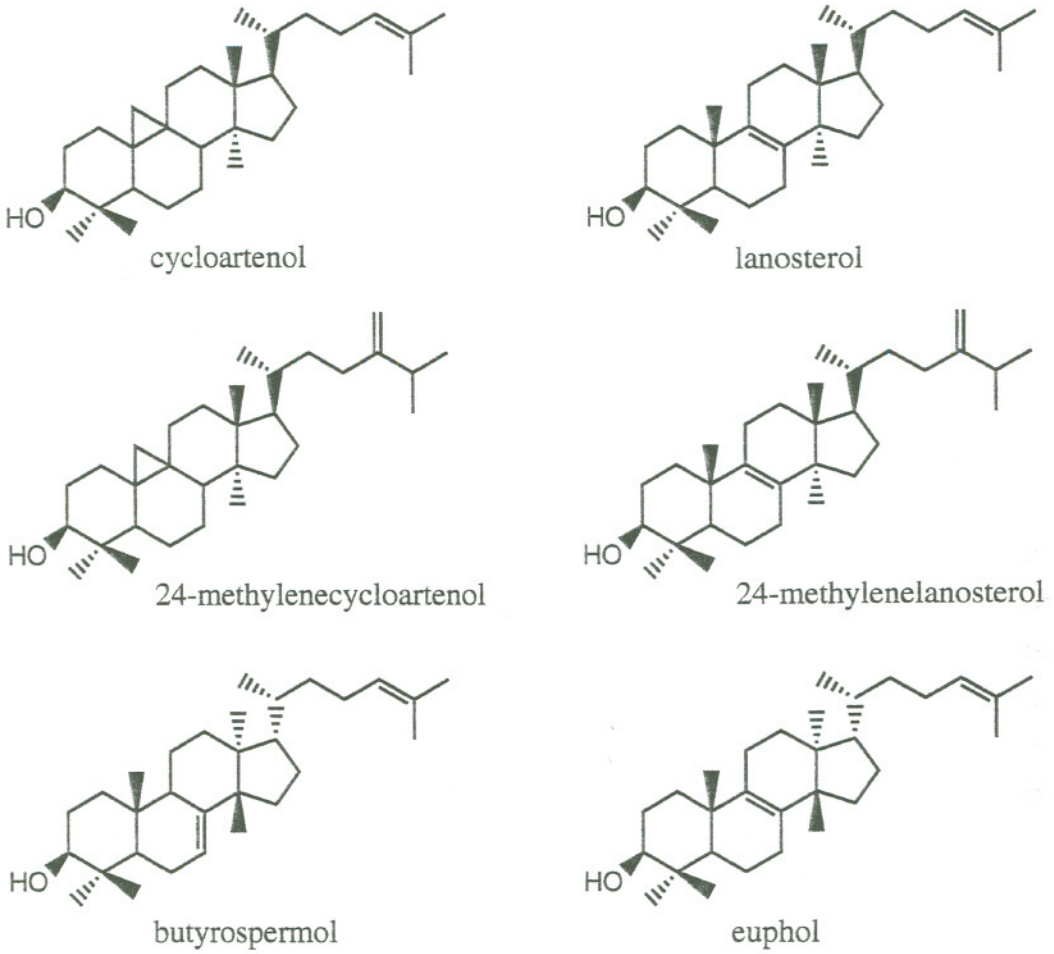

Figure 4. Major triterpenoid compounds found in the latex of the *Euphorbia lathyris* plant.

Despite finding no evidence of *de novo* fatty acid synthesis occurring in the latex, Nemethy did determine that the acyl donor for the conversion of the free triterpenols to their fatty acid ester analogs was a phospholipid, phosphatidylcholine. The esterification was found to be enzyme-catalyzed. Furthermore, she initially concluded that the triterpenol pool and the ester pool were synthesized in independent pathways, though later evidence suggested that the triterpenol was synthesized in a single route, and then esterified in a step requiring a soluble cofactor.

Experiments with isolated latex and varying amounts of S-adenosyl methionine (SAM) indicated that SAM was the required soluble factor. SAM was needed as a methyl donor in two sets of crucial reactions: the synthesis of the acyl donating phosphatidyl-choline from phosphatidyl-ethanolamine requires three SAM molecules; and the formation of two of the terpenols, 24-methylene cycloartenol and 24-methylene lanosterol, requires SAM for the conversion of the C_{30} starting compounds to the final C_{31} products [Skrukrud et. al 1987].

To determine the subcellular organization of the triterpenol biosynthetic pathway in latex, a set of experiments were undertaken with isolated latex, combining assays with radiolabeled substrates, subcellular fractionation by differential sedimentation followed by isopycnic centrifugation on Percoll gradients, and electron microscopy analysis of the fractionated subcellular particles (Figure 5). These experiments were initiated by Nemethy, Skrukrud and Hawkins, who were later joined by Dr. Scott Taylor. They found that the pathway from acetate to triterpenol could be subdivided into two separate fractions: the steps from acetate to mevalonic acid remained free in the latex solution, and the conversion of mevalonic acid to the triterpenols was associated with an organelle [Skrukrud et. al 1989]. Electron micrographs of this subcellular structure (Figure 6) showing a single bounding membrane and biochemical marker studies showing the presence of mannosidase indicated that this organelle was a vacuole. Once synthesized, the triterpenols and their esters are stored in a separate organelle or particle that binds to polylysine, indicative of a negatively-charged membrane surrounding the pool of triperpenols. No triterpenol biosynthetic activity was found to be associated with starch grains nor any other plastid-like structure.

A parallel set of experiments was undertaken by Skrukrud to identify the rate-limiting steps in the pathway of terpenol biosynthesis [Skrukrud thesis 1987]. She was able to determine that the rate of incorporation of mevalonate into triterpenols was 30 times greater than that of acetate, indicating that the rate of triterpenol biosynthesis was limited prior to mevalonate conversion. The enzyme responsible for the conversion of hydroxymethylglutarate (HMG) to mevalonate, HMG-CoA Reductase (HMGR), was found to be associated with a subcellular structure. The rate of HMG to mevalonate conversion was found to be identical to the acetate to mevalonate conversion rate, indicating that the step catalyzed by HMGR limits the

rate of production of triterpenols in the *E. lathyris* latex. The pH optimum of the latex HMGR, 5.8, was suggestive of a vacuolar localization.

Figure 5. Scanning electron micrograph of the subcellular particles found in centrifuged *Euphorbia lathyris* latex. Bar equals 10 μm.

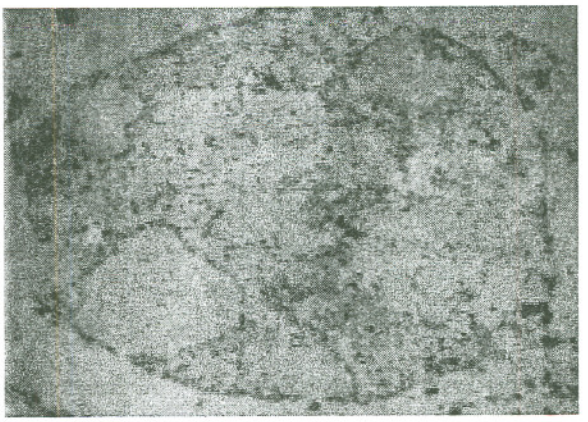

Figure 6. Transmission electron micrograph of the triterpenoid-synthesizing structure found in *E. lathyris* latex. This subcellular structure is approximately 6 μm across.

Skrukrud also found HMGR activity in the leaves of *E. lathyris*, though the majority of activity was associated with the plastids, and the highest specific activity was found in the mitochondria. The pH maximum for the leaf forms of the enzyme was observed to be about 6.8. She concluded that at least two and possibly 3 or more isozymes of HMGR exist in *E. lathyris*, an important fact to remember if one attempts, through genetic engineering methodology, to increase triterpenol biosynthesis by manipulation of the latex HMGR.

Applied environmental stress has been shown to increase secondary plant product, including isoprenoid, formation in numerous plants. Taylor and Skrukrud wondered if this was the case for *E. lathyris* [Taylor et. al 1989]. Plants were grown in hydroponic solution, and salinity stress (added NaCl) was applied to the plant. Results indicated that low level salinity (50 mM NaCl) resulted in a 50% increase in terpenoid production with minimal changes in growth pattern. Associated with this increase in triterpenol synthesis was an 85% increase in whole plant HMGR activity. These results indicated that the controlled application of stress at the right time of the growth cycle could result in larger hydrocarbon yields, and that HMGR activity was associated with these increases.

As part of his thesis project, Hawkins studied another important step in the terpenol synthesis pathway, the cyclization of squalene to form the polycyclic triterpenols of *E. lathyris*. Central to his study was the question of which route was taken to form to two initial products, lanosterol and cycloartenol [Hawkins et. al 1991]. In almost all non-photosynthetic organisms, the initial product of the cyclization of the 2,3-squalene epoxide is lanosterol. In plants, the product of this reaction is usually cycloartenol. As stated previously, both lanosterol and cycloartenol are found in the latex. Therefore, the question was posed as to whether cycloartenol or lanosterol was the initial product, followed by conversion from one form to the other, or were these two molecules instead produced by two separate and distinct enzymatic processes.

Hawkins used the morpholine-based fungicides tridemorph and fenpropimorph with isolated latex to follow squalene cyclization. Tridemorph inhibits both general cyclization and ring opening, and had a greater effect on cycloartenol than on lanosterol. Fenpropimorph inhibits ring opening but not cyclization, and had no effect on synthesis of either compound. The results were interpreted to indicate that lanosterol could not have been derived from cycloartenol, since that conversion would require a ring opening. The other possible reaction, the conversion of lanosterol to cycloartenol could not be excluded, but seemed unlikely in the light of research of other groups with dual isotope labeling experiments that have shown that cycloartenol is not derived from lanosterol. Hawkins concluded that the two paths are separate.

III. Algae as a Source of Hydrocarbons

Calvin's interest in algal hydrocarbons [McCarthy and Calvin 1967, Han et. al 1968ab, 1969] predated his attempt to find renewable plant-derived energy sources. He was first attracted to the alga cell hydrocarbon because of a basic curiosity- by what biosynthetic mechanism would an alkane be created in a cell. It wasn't until later that he saw this mechanism as a possible means of converting common algal constituents into an easily utilized fuel.

The use of algae as an energy source has strong advantages. Algae have adapted to a wide range of environmental conditions, and are thus able to survive at high temperature in water with a high salt content. These qualities would make them

ideal candidates for aquatic energy farms located in arid, non-agricultural regions, employing low quality, brackish water. In addition, some species have the ability to fix (or reduce) N_2 into biological molecules, removing the need for providing nitrogen additives to the culture solution.

A number of microalgae are capable of producing significant quantities of highly reduced hydrocarbon-like materials. These are derived from one of two separate biosynthetic routes: fatty acid/lipid synthesis or the isoprenoid pathway. The potential of modifying these algae to increase yields so that they may be a commercially-viable source for alternative fuels was studied in Calvin's laboratories, starting with the work of Jerry Han in 1968. Certain algal species have been reported to contain lipid levels of up to 72% of their dry weight, or isoprenoid constituents of up to 40% of dry weight. The successful combination of these hydrocarbon-producing pathways in balance with the cellular mechanisms involved in rapid growth and wide environmental tolerances was an early interest of Calvin's plant hydrocarbon group. Of special interest was the production of alkanes and isoprenoids, which are handled easier than lipids and in many cases are more reduced and thus more energy-rich.

Han and co-workers identified and quantified the hydrocarbon constituents of four cyanobacteria species, *Anacystis nidulans, Chlorogloea fritschii, Nostoc moscorum* and *Phorimidium luridum* and chlorophyta, a *Spirogyra* and a *Chlorella* species. They found small amounts of alkanes and alkenes, predominately n-heptadecane, n-heptadecene, n-pentadecane and branched 18-carbon molecules, in the range of 0.002-0.035% of the cell dry weight [Han et al 1968ab].

Further study elucidated the biosynthetic route of these aliphatic compounds in *Nostoc moscorum.* The unbranched compounds were found to be derived from the enzyme-catalyzed decarboxylation of fatty acids. The branched alkanes, consisting of an equimolar distribution of 7- and 8-methylheptadecanes were created in a two-step process: methylation of a double bound in the fatty acid followed by the decarboxylation step [Han et. al 1969]. The fact that a relatively abundant cellular constituent, lipid, could be converted to the preferable alkanes via a one-step, enzyme-catalyzed reaction led Calvin and Taylor [1989] to propose this decarboxylation enzyme as a good candidate for use in genetic manipulation experiments to convert high lipid-producing species to alkane-producing clones.

A second area of interest in algal-derived fuels centered around the green alga *Botryococcus*. Previous work found that up to 86% of the dry weight of the cell of this species was hydrocarbons, and consisted of a mixture of long chain linear alkenes (C_{27}, C_{29}, C_{31} dienes) and branched isoprenoids, called botryococcenes. Dr. Fred Wolf joined with Dr. James A Bassham and Calvin in 1985 to study the biosynthetic pathways involved in the production of these two groups of algal hydrocarbons and to further identify the isoprenoid constituents. He found these botryococcenes to be C_{30}-C_{37} isoprenoids, with a general formula of C_nH_{2n-10} [Wolf et. al 1985a]. Wolf also reported that these algae existed in one of two different metabolic states, one with linear alkenes production (via fatty acid synthesis, elongation and decarboxylation) and the other favoring botryococcene production (Figure 7) [Wolf et. al 1985a]. Highest overall hydrocarbon yields were produced when isoprenoid synthesis was active. The mechanism that controls carbon partitioning to these two distinct metabolic pathways is not yet understood, and many laboratories have been unable to obtain isoprenoid production under controlled culture conditions, limiting the economic exploitation of this alga.

Figure 7. Hydrocarbon production by colonies of *Botryococcus braunii* algae.

IV. Hydrocarbon Content of Other Plant Species

Other higher plant species in addition to *E. lathyris* were also screened for hydrocarbon content in the search for an agronomic energy crop. Calvin and Dr. Hiroyuki Nishimura [Nishimura and Calvin 1979; Nishimura et. al 1980] examined the composition of oils extracted from *Eucalyptus globulus* and *Eucalyptus radiata* as potential sources of renewable fuels. They observed that the extractable oils were rich in mono- and sesquiterpenes such as α-pinene, piperitone, α-terpineol, 1,8-

cineol, aromadendrene and globulol, all compounds with high energy content. The limitation on the economic utilization of *Eucalyptus* as a fuel stock was the relative low growth rate of the trees and the low oil content, ranging from about 1-6% of the fresh weight of the shoots, leaves and fruit.

Nemethy, Hawkins and Dr. Regina Lago looked at the constituents of the fruit from the Panamanian *Myristica otoba* tree. Extraction of the fruit in dichloromethane yielded a viscous oil, which constitutes 74% of the fruit's fresh weight. This oil could be further separated into a neutral fraction (14% of the oil) and a triglyceride fraction (86%). Further solvent separations of the neutral fraction, followed by HPLC separation and analysis by proton NMR, mass spectroscopy and CD spectroscopy, resulted in the purification and identification of seven aryletrailin-type lignans [Nemethy et. al 1986]. The triglycerides were hydrolyzed, methylated, and analyzed via gas chromatography-mass spectroscopy. The fatty acids ranged from C_{10}-C_{18}, with the major constituent being myristic acid (14:0), a compound in high demand by the detergent industry.

Nemethy and Lago also examined the composition of oils from the fruit of the Macuaba palm (*Acrocomia sclerocarpa*). The fruit were separated into pulp and kernel oils. Over 90% of the oils were found to be triglycerides of lengths from 10 to 20 carbons. The major constituent of the pulp oil was oleic acid (18:1), while the major components of the kernel oil were lauric (12:0) and oleic acids.

Nemethy and Taylor studied the hydrocarbon content of the fruit from the *Pittosporum resiniferum* tree. Utilizing steam distillation, a hydrocarbon fraction (2% of fruit fresh weight) was collected and analyzed by gas chromatography. The major constituents were free alkanes (heptane and nonane) and monoterpenes (α-pinene, ß-pinene and myrcene).

In 1987 Dr. Dolon Konwer of the Assam Agricultural University in Jorhat, India took sabbatical leave and joined Calvin's group to examine the potential of using a seed oil from a tree local to Assam for production of fuel. Oil from the seeds of the *Mesua ferrea* tree was extracted by a solvent-Soxhlet process, and analyzed by gas chromatography. Up to 75% of the weight of the seeds was found to be oil, most of which were triglycerides. The major esters were those of oleic, linoleic, palmitic and stearic acids. These fatty acids were then pyrolyzed in the presence of sodium

carbonate to convert the fatty acids to a combination of aromatics, olefins and saturated compounds. Gas chromatographic-mass spectroscopic analysis showed that the saturated compounds were primarily normal alkanes of lengths from 6 to 17 carbons [Konwer et. al 1989].

V. Conclusion

A logical extension of his early work on the path of carbon during photosynthesis, Calvin's studies on the production of hydrocarbons by plants introduced many in the scientific and agricultural worlds to the potential of renewable fuel and chemical feedstocks. He and his co-workers identified numerous candidate compounds from plants found in tropical and temperate climates from around the world. His travels and lectures concerning the development of alternative fuel supplies inspired laboratories worldwide to take up the investigation of plant-derived energy sources as an alternative to fossil fuels.

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VII. Chronological Bibliography of Calvin's Work on Hydrocarbon-producing Plants

Publications

McCarthy ED, M Calvin. Organic geochemical studies, I. Molecular criteria for hydrocarbon genesis. *Nature* **216**: 642 (1967)

Han J, ED McCarthy, W van Hoeven, M Calvin, WH Bradley. Organic geochemical studies, II. A preliminary report on the distribution of aliphatic hydrocarbons in algae, in bacteria, and in a recent lake sediment. *Proc Nat Acad Sci USA* **59**: 29 (1968a)

Han J, ED McCarthy, M Calvin, MH Benn. Hydrocarbon constituents of the blue-green algae *Nostoc muscorum, Anacyctis nidulans, Phorimidium luridum* and *Chlorogloea fritschii. J Chem Soc* **C**: 2785 (1968b)
Han J, HWS Chan, M Calvin. Biosynthesis of alkanes in *Nostoc muscorum. J Amer Chem Soc* **91**: 5156 (1969)
Calvin M. Solar energy by photosynthesis. *Science* **184**:375 (1974a)
Calvin M. Solar energy by photosynthesis: are we able to raise enough cane to get it? *Kagaku to Seibutsu* **12**: 481 (1974b)
Calvin M. Solar energy by photosynthesis In: *Energy: Use Conservation and Supply,* PH Abelson (ed), AAAS, Washington DC (1974c), pp111-117
Calvin M. Photosynthesis as a resource for energy and materials. *Kemie e Kemi* **2**: 46 (1975)
Calvin M. Photosynthesis as a resource for energy and materials. *Amer Scientist* **64**: 270 (1975)
Calvin M. Photosynthesis as a resource for energy and materials. *Photochem Photobiol* **23**: 425 (1976a)
Calvin M. Photosynthesis as a resource for energy and materials In: *The Recent History of Natural Products, Including Tobacco,* NJ Fina (ed), Phillip Morris, Inc, New York (1976b), pp 62-99
Calvin M. Energy and materials via photosynthesis In: *Living Systems as Energy Converters,* R Buvet et. al (eds), Elsevier-North-Holland Biomedical Press, Amsterdam (1977a) pp 231-259
Calvin M. The sunny side of the future. *Chem Tech* **7**: 352 (1977b)
Calvin M. Hydrocarbons via photosynthesis. *Energy research* **1**: 299 (1977c)
Nielsen P, H Nishimura, JW Otvos, M Calvin. Plant crops as a source of fuel and hydrocarbon-like materials. *Science* **198**: 942 (1977)
Nishimura H, RP Philip, M Calvin. Lipids of *Hevia brasiliensis* and *Euphorbia coerluscens. Phytochem* **16**: 1048 (1977)
Calvin M. Hydrocarbons via photosynthesis In: *Energy and Chemical Sciences,* Pergamon Press, New York (1978a) pp 1-30.
Calvin M. Hydrocarbons via photosynthesis In: *Guayule,* E. Campos-Lopez (ed), Centro de Investigacion en Quimica Applicada, Saltillo, Mexico (1978b) pp 103-150
Calvin M. Chemistry, population, resources. *Pure Appl Chem* **50**: 407 (1978c)
Calvin M. Fermentation and hydrocarbons In: *Bio-Energy: Energy from Living Systems,* Gotlieb Duttweiler Institut, Roschlikon, Zurich, Switzerland (1978d) pp 89-117.
Calvin M. Petroleum plantations In: *Energy Technology V,* RF Hill (ed), Government Institutes, Inc., Washington, DC (1978e) pp 687-705.
Calvin M. Chemistry, population, resources. *Interdis Sci Res* **3**: 233 (1978f)
Calvin, M. Green Factories. *Chem Rng News* **50**: 30 (1978g)
Calvin M. Petroleum plantations and synthetic chloroplasts. *Energy* **4**: 851 (1979a)
Calvin M. Petroleum plantations for fuel and materials. *BioScience* **29**: 533 (1979b)
Calvin M. Fuel from plants. *SciQuest* **52**: 16 (1979c)
Calvin M. Petroleum plantations In: *Solar Energy, Chemical Conversion and Storage,* RR Hautala, RB King, C. Kutal (eds), Humana Press (1979d) pp 1-30
Calvin M, G Calvin. Plant power. *The Sciences* **19**: 24 (1979)

Nemethy EK, JW Otvos, M Calvin. Analysis of extractables from one Euphorbia. *J Amer Oil Chem Soc* **56**: 957 (1979)
Nielsen PE, H Nishimura, Y Liang, M Calvin. Steroids from Euphorbia and other latex-bearing plants. *Phytochem* **18**: 103 (1979)
Nishimura H, M Calvin. Essential oil of *Eucalyptus globulus* in California. *J Agric Food Chem* **27**: 432 (1979)
Calvin M. Hydrocarbons from plants: analytical methods and observations. *Die Naturwissen* **67**: 525 (1980)
Nishimura H, DM Paton, M Calvin. *Eucalyptus radiata* oil as a renewable biomass. *Agric Biol Chem (Japan)* **44**: 2498 (1980)
Bissell MJ, EK Nemethy, L Riddle, M Calvin. Testing for tumor promoters in *Euphorbia lathyris*: analysis of possible health hazards. *Bull Environ Contamin Toxicol* **27**: 894 (1981)
Calvin M, EK Nemethy, JW Otvos, K Redenbaugh. *Euphorbia lathyris*: plants can be a direct source of fuel. *Petroculture* **2**: 26 (1981)
Nemethy EK, JW Otvos, M Calvin. Hydrocarbons from *Euphorbia lathyris*. *Pure Appl Chem* **3**: 1101 (1981a)
Nemethy EK, JW Otvos, M Calvin. Natural production of high-energy liquid fuels from plants In: *Fuels from Biomass*, DL Klass, GH Emert (eds), Ann Arbor Science Publishers (1981b) pp 405-419.
Calvin M. Petroleum plantations. *Proc 1st Asian Pacific Chem Cong* (1982a) pp 1-15
Calvin M. Renewable fuels for the future In: *Energy Resources an Environment*, SW Yuan (ed) Pergamon Press, New York (1982b) pp 11-21
Calvin M, EK Nemethy, K Redenbaugh, JW Otvos. Plants as a direct source of fuel. *Experietia* **38**: 18 (1982a)
Calvin M, EK Nemethy, K Redenbaugh, JW Otvos. Plants can be a direct fuel source. *Biologist* **29**: 145 (1982b)
Nemethy EK, M Calvin. Terpenes from *Pittosporaceae*. *Phytochem* **21**: 2987 (1982)
Calvin M. New sources for fuel and material. *Science* **219**: 24 (1983a)
Calvin M. The path of carbon: from stratosphere to cell In: *Advances in Gene Technology: Molecular Genetics of Plants and Animals*, F Ahamd, K Downey, J Schultz, RW Voellmy (eds), Academic Press, New York (1983b) pp 1-35.
Nemethy EK, Calvin M. Terpenoid biosynthesis in *Euphorbia lathyris* In: *Biosynthesis and Function of Plant Lipids*, JB Mudd, M Gibbs (eds), Amer Soc Plant Physiol, Rockville, MD (1983) pp 216-228.
Nemethy EK, C Skrukrud, GJ Piazza, M Calvin. Terpenoid biosynthesis in Euphorbia latex. *Biochim Biophys Acta* **760**: 343 (1983)
Calvin M. Renewable fuels for the future. *J Appl Biochem* **6**: 3 (1984a)
Calvin M. Oil from plants In: *Handbook of Plant Cell Culture Vol. 2*, WR Sharp, DA Evans, PV Ammirato, Y Yamato (eds) MacMillian Publishing Co, New York (1984b) pp 1-18.
Nemethy EK. Biochemicals as an energy resource. *CRC Crit Rev Plant Sci* **2**: 117 (1984)
Calvin M. Renewable fuels for the future In: *Alternative Sources of Energy for Agriculture*, J Bay-Petersen (ed), Food and Fertilizer Technol Center, Taiwan (1985a) pp 1-17

Calvin M. Fuel oils from higher plants. *Ann Proc Phytochem Soc Eur* **26**: 147 (1985b)
Calvin M. Renewable resources for fuel and materials In: *Energy Applications of Biomass*, MZ Lowenstein (ed), Elsevier, New York (1985c) pp3-11
Wolf FR, AM Nonomura, JA Bassham. Growth and branched hydrocarbon production in a strain of *Botryococcus braunii* (Chlorophyta). *J Phycol* **21**: 388 (1985a)
Wolf FR, EK Nemethy, JH Blanding, JA Bassham. Biosynthesis of unusual acyclic isoprenoids in the alga *Botyrococcus braunii*. *Phytochem* **24**: 733 (1985b)
Calvin M. Renewable fuels and materials: oils from plants. *Cell Biophys* **9**: 189 (1986)
Nemethy EK, R Lago, D Hawkins, M Calvin. Lignans from *Myristica otoba*. *Phytochem* **25**: 959 (1986)
Calvin M. Fuel oils from euphorbs and other higher plants. *Bot J Linnean Soc* **94**: 97 (1987a)
Calvin M. High energy fuels and materials from plants. *J Chem Educ* **64**: 335 (1987b)
Hawkins DR, M Calvin. A simple, efficient synthesis of 3'-$^2H_3$ mevalonic acid. *Radiopharm* **24**: 1229 (1987)
Skrukrud CL, SE Taylor, DR Hawkins, M Calvin. Terpenoid biosynthesis in *Euphorbia lathyris* In: *Plant Lipids: Biochemistry, Structure and Function*, PK Stumpf, JB Mudd, WD Nes (eds), Plenum Press, New York (1987) pp 115-118.
Taylor SE, M Calvin. Hydrocarbons from plants: biosynthesis and utilization. *Comments Agric Food Chem* **1**: 1 (1987)
Skrukrud CL, SE Taylor, DR Hawkins, EK Nemethy, M Calvin. Subcellular fractionation of terpenoid biosynthesis in *Euphorbia lathyris*. *Physiol Plant* **74**: 306 (1988).
SE Taylor, CL Skrukrud, M Calvin. Effect of salinity on the allocation of carbon to energy-rich compounds in *Euphorbia lathyris* In: *Energy from Biomass and Wastes XI*, DL Klass (ed), Institute of Gas Technology, Chicago (1988) pp 903-916.
Calvin M. Intermediates in the photosynthetic cycle: an essay. *Biochim Biophys Acta* **1000**: 403 (1989a)
Calvin M. Forty years of photosynthesis and related activities. *Photosyn Res* **21**: 1 (1989b)
Calvin M, SE Taylor. Fuels from algae In: *Algal and Cyanobacterial Biotechnology*, RC Creewell, TAV Rees, N Shah (eds), Longmans, London (1989) pp 137-160
Konwer D, SE Taylor, BE Gordon, JW Otvos, M Calvin. Liquid fuels from *Mesua ferrea* L. seed oil. *J Amer Oil Chem Soc* **66**: 223 (1989).
Hawkins DR, SE Taylor, M Calvin. The formation of lanosterol and cycloartenol in *Euphorbia lathyris* L. latex. *Life Sci Advances (Plant Phys)* **10**: 151 (1991)

Technical Reports-LBNL

Calvin M. Hydrocarbons via Photosynthesis. LBL 5378, Sept. 1976
Calvin GJ, M Calvin. The Only Source of Energy. LBL 7548, March 1978
Calvin GH, M Calvin. Green Factories for Liquid Fuels. LBL 7584, April 1978
Nemethy EK, JW Otvos, M Calvin, R Sachs. Hydrocarbons and Energy from Plants. LBL 8596, Nov. 1978

Karenzi PC, EK Nemethy, JW Otvos, M Calvin. Latex-producing Plants from Rwanda. LBL 14212, Jan. 1982
Calvin M. Energy Sources and Climate. LBL 14496, May 1982
Calvin M. Hydrocarbons from Plants and Trees. LBL 14905, Aug. 1982
Calvin M, Energy Agriculture. LBL 15168, Sept. 1982
Calvin M. Oil from Plants. LBL 15678, Jan. 1983
Sonola T, EK Nemethy, M Calvin. Cassava- A Latex-bearing Plant from Nigeria. LBL 16223, June 1983
Otvos JW. Energy Content of Biomass: Calculation from Elemental Composition. LBL 22916, Jan. 1987

Technical Reports- Oak Ridge National Laboratory

Calvin M, JW Otvos, SE Taylor, EK Nemethy, CL Skrukrud, DR Hawkins, R Lago. Hydrocarbons and Energy from Plants: Final Report 1984-1987. ORNL/Sub 84-90435/1. August 1988

PhD Thesis

Skrukrud, Cynthia Louise. Terpenoid Biosynthesis in *Euphorbia lathyris* and *Copaifera* spp. (Group in Comparative Biochemistry) LBL 23915, August 1987
Hawkins, Douglas Raymond. Triterpenoid Biosynthesis in *Euphorbia lathyris* Latex. (Chemistry) LBL 24352, December 1987

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