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NEW SOURCES FOR FUEL AND MATERIALS

Melvin Calvin

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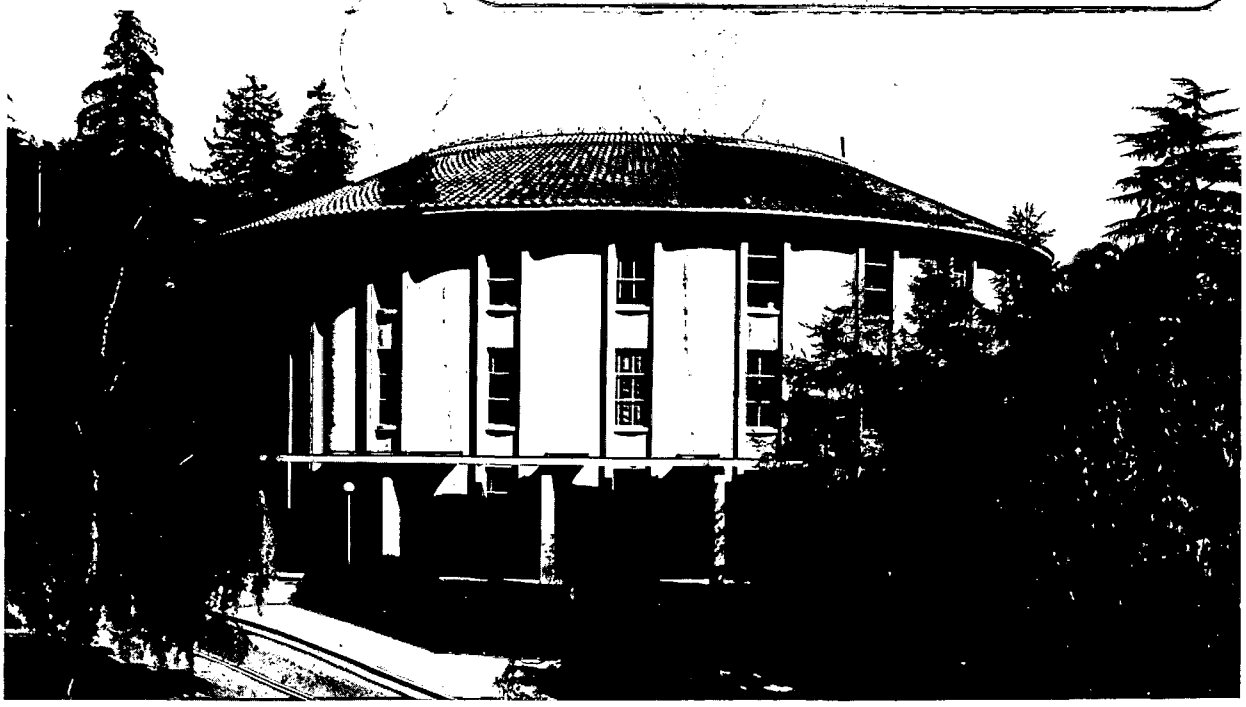

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NEW SOURCES FOR FUEL AND MATERIALS

Melvin Calvin

Department of Chemistry and Lawrence Berkeley Laboratory

University of California, Berkeley, California 94720

Abstract: A selection of new plant sources with high potential for production of chemicals and liquid fuels is reviewed. Some existing productivity data is given and suggestions are made for modification of both the product character and the productivity of the plants.

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Introduction

Shortly after the 1973 oil embargo we began our efforts to find plants that could produce liquid fuel which could be used directly (1). To initiate the study of using green plants as alternate energy sources we went to Brazil where they have developed processes for using sugar cane for producing alcohol for fuel additives. In 1974 the production of "gasohol" was 400 million liters of alcohol and by 1981 the production was 4.4 billion liters of fermentation alcohol. In actuality, the alcohol produced from the sugar cane in an enclosed system may be more useful as a chemical intermediate than as a fuel for automobiles. Similarly, in Puerto Rico where sugar cane has been grown for its carbohydrate production, efforts have been made to improve the plant for higher cane production (2). This so-called "energy" cane (Fig. 1) produces about 250 tons/hectare of total biomass, but the sugar content per hectare is the same for both "energy" cane and ordinary sugar cane. The energy cane has three times as much product which can be used, for example, to fire power boilers to produce electricity.

Isoprenoids from the Whole Plant

Most of the plants we have examined in any detail belong to the family *Euphorbiaeceae,* which is the same family to which the rubber tree (*Hevea brasiliensis*) belongs. Various species of Euphorbias grow in all types of climate throughout the world, but we emphasized those which would grow on land not suitable for food production. The plant we have studied in greatest detail is *Euphorbia lathyris* and experimental plantations were developed in California (northern and southern) as well as on semiarid land near the University of Arizona (3-6). The conceptual processing sequence to recover fuels and materials from *E. lathyris* is simple and related to that used to extract seeds and oils (7). The first step, after

the plant is ready for harvest, is cutting and drying. After solvent extraction of 1000 tons of the dried plant material, the product is 8 tons of oil. After the first extraction (essentially a soybean oil type of extraction process using hexane), we then extract sugar using aqueous methanol, resulting in 200 tons of fermentable sugar. The bagasse which remains can be used to run the entire process, i.e., to make steam for the extraction process, with another 200 tons of bagasse left over which could be used to distill the alcohol, if alcohol were the final product of the sugar. The material balance for 1000 dried tons per day of E. lathyris therefore is: 8 tons of oil, 200 tons of sugar and another 200 tons of bagasse over and above the amount required to run the extraction process itself.

The oil obtained from E. lathyris after extraction (8) has been subjected to catalytic cracking using the special zeolite catalysts developed by Mobil Oil Corporation (9). The usual suite of products results: Ethylene (10%), propylene (10%), toluene (20%), xylenes (15%), C_{5-20} non-aromatics (21%), coke (5%), C_{1-4} alkanes (approx. 10%) and fuel oil (about 10%). All of these materials are useful for petrochemical industrial processes.

The main thrust of the discussion is, however: How does the green plant make these materials and is there anything we can do to "improve" the yield of oil, not only in quantity but also the various components of the oil. The biosynthetic route by which the plant makes the oil is fairly well known, coming from sugar via the glycolytic cycle to get to pyruvate, which is then built up to mevalonic acid and goes on to give isoptentenyl-pyrophosphate (IPP). The IPP goes on further to polymerize a variety of isoprenoids. Normally, in the Euphorbia lathyris the material goes on through the isoprenoid biosynthetic pathway to squalene (C_{30}) which is then folded

up to make the C_{30} terpenoid alcohols which constitute the greater percentage of the oil. In actuality, all the Euphorbias take that route.

Isoprenoids by Tapping the Tree

It turns out that there is in another plant family in Brazil, the Leguminosae, a genus called Copaifera which contains trees which are prolific terpene producers. The product of the Copaifera multijuga, for example, is a sesquiterpene. This particular tree (Fig. 2) is harvested by drilling a hole in the trunk about 3 ft from the ground; the hole is about 2 cm in diameter and goes into the heart wood of the tree. A pipe is inserted in the hole, and the oil drains out of the pipe into a bucket. This operation can be done twice each year, and in 24 hrs about 20 liters of material, similar to diesel fuel, accumulates. The hole is then plugged with a bung, and 6 months later the tree will produce another 20 liters from the same hole. The oil comes not from the cambium, as does the rubber latex in the H. brasiliensis, but from the heart wood, from pores (1-2 mm in diameter) running vertically throughout the trunk of the tree. There are at least twenty-five different compounds in the oil from these trees (called Copaiba oil) which have been analyzed by gas-liquid chromatography and each compound is a C_{15} sesquiterpene (3).

An experimental plantation of C. multijuga is being developed in the Ducke Forest in Manaus, Brazil. The purpose is to try and understand the mechanism of the diesel formation in the trees, with the possibility of perhaps increasing the yield of this material. Agronomic studies are underway as well to see whether or not the C. multijuga could be established as a viable commercial crop. Also, the question of whether or not it is possible to use more than one tap in each tree has not yet been answered satisfactorily.

The biosynthetic method by which the diesel oil from the Copaifera is made is the same as that used by the Euphorbia lathyris up to the C_{15} point. The Copaifera cyclizes the C_{15} farnesyl pyrophosphate, that is, drops the phosphorus off to give the cyclic C_{15} compounds. One type of enzyme is responsible for the difference in the two end products of C. multijuga and E. lathyris. In the case of the E. lathyris this enzyme (farnesyl pyrophosphate) is dimerized, whereas with the Copaifera the material is cyclized with many C_{15} products as a result. The oil from the Copaifera is used for medicinal purposes by Amazon natives, it is a component in pharmaceutical products and is also used directly as a fuel in automobiles.

Isoprenoids from Fruits and Seeds

There are a number of other plant species whose oil has come to our attention. One, Pittosporum resiniferum (10), grows in the Philippines. The fruit of this plant is quite large and is used frequently as a source of illumination by tying it to the end of a stick and lighting it. We analyzed some of the "oil" from these fruits (called petroleum nuts) and the preliminary results show that the major products are α-pinene (38%), myrcene (40%), n-nonane (3%) and heptane (5%) (11). This fruit has terpenes not glycerides in it. However, another species of Pittosporum growing in California (P. undulatum) gave slightly different results, with the fruits being much smaller. The major products from these fruits turned out to be α-pinene and limonene.

We have found some other sources as potential fuel and material candidates. These are: Marmeleiro (Euphorbiaceae, Croton) from Brazil; Andiroba (Carapa guiarensis) also from Brazil; the Copaiba sesquiterpenes (Copaifera multijuga) from Brazil; monoterpenes from Pittosporum undulatum

(California); and monoesters from Jojoba. Four of these oils are terpenes which have the desired characteristics for fuel and materials; one, Jojoba, is a monoester; and one, Andiroba, is a triglyceride similar to soybean oil, olive oil and palm oils. Most seed oils are glycerides and some are so saturated that they solidify at room temperature. Various experiments are underway to use vegetable and seed oils as diesel substitues, particularly in farm machinery. In our search we have found three different kinds of oils, chemically: Terpenes (E. lathyris, P. undulatum, P. resiniferum, Copaiba), monoesters (Jojoba) and triglyderides (Andiroba).

Gene Transfer Possibilities

Can we transfer the gene for the production of sesquiterpenes from the Copaifera multijuga (or other Copaifera species) to such plants as Euphorbia lathyris? It is not possible to grow Copaifera in the United States and gene transfer seems to be one way of using the characteristics of that species in another. As mentioned earlier, there is only a single enzyme, farnesyl pyrophosphate, involved to move the C_{15} pyrophosphate into cyclic C_{15} instead of having the compounds go all the way to C_{30}. In other words, a single gene transplant from the donor cell of the Copaifera to the acceptor cell of Euphorbia lathyris (12) would be required. It is, however, necessary to find a donor cell which has the genes for the enzyme we want, get the messenger out, make a copy of the DNA, insert the copy DNA into a plasmid, clone the plasmid in E. coli and then, by means of the plasmids, insert the gene into a selected plant such as Euphorbia lathyris. Eventually, the piece of genetic information can be integrated into the nuclear gene of the transformed cell. This has been done with bacteria but has not yet been accomplished with higher plants as the many problems involved in performing gene transplants with eukaryotic plants have not yet been solved.

Tissue Culture and Plantlet Regeneration

The materials with which we have been working to achieve such gene transfers are the donor plant (a species of Copaifera, Fig. 3) and the acceptor plant (E. lathyris). The first step is to prepare a tissue culture of the acceptor plant cell from E. lathyris and this process has resulted in a callus (Fig. 4) (13). The E. lathyris leaf mesophyll protoplasts have aggregated to the callus (Fig. 5) (14), getting shoots from the protoplasts and eventually roots. We have devised a method for selecting E. lathyris protoplasts using the technique of cell sorting (15), a mechanical selection procedure. This method depends on the cells flowing past a laser beam with a number of light detectors around the area. One cell is stained with a material (fluorescein) that fluoresces yellow and to the other cell is applied a stain (rhodamine) which fluoresces red. If cell fusion occurred, there would be a cell that fluoresces in two colors and it should then be possible to select the cells that have the double color. We have been able to select the few fused cells away from the unfused parent cells. Therefore, it now seems possible to use a mechanism of genetic manipulation at the somatic level without interfering with the germ plasm, somatic hybridization, in other words.

This idea is introduced to give some sense of what will be possible in plant breeding using the new technology of genetic engineering. There are many uncertainties in manipulations of this type, but there is no question that this technology will be increasingly important. We have learned (1) to separate and fuse cells, but have not yet introduced any new genes into the cells, and (2) we have been able to regenerate plants from tissue culture. However, we have not yet regenerated a shoot or even a callus from a fused protoplast.

Conclusion

It is possible to use the green plant, the best solar energy capturing device we know, to produce the materials we need, namely, hydrocarbons of suitable molecular weight and structure. This can be done by plant selection and modification, both by classical plant breeding and by using the newer techniques of genetic engineering and plant tissue culture. The choice of plants will depend on agronomic characteristics, hydrocarbon productivity, harvestability and process development. Studies have indicated the feasibility of using hydrocarbon producing plants for energy agriculture. Next steps in the development will be a refinement of tissue culture and plant cloning techniques to introduce suitable enzymes from one plant to another to produce the chemicals most useful for fuels and materials and, finally, to build the pilot plants to extract and process the oil(materials) for use.

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14. Photograph by S. Ruzin, Melvin Calvin Laboratory, University of California, Berkeley.

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16. This paper was presented at the symposium and workshop on "Biomass Substitutes for Liquid Fuels", Campinas, Brazil, February 9-12, 1982.

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Figure Captions

Figure 1 "Energy" cane (left), Puerto Rico. Photo by Gene Elle Calvin.

Figure 2 *Copaifera multijuga*, Ducke Forest, Manaus, Brazil showing bung in trunk. Photo by George Ancona

Figure 3 Copaifera seedlings, Melvin Calvin Laboratory, University of California, Berkeley.

Figure 4 *Euphorbia lathyris* callus, Melvin Calvin Laboratory, University of California, Berkeley; K. Redenbaugh.

Figure 5 *Euphorbia lathyris* shoots from leaf protoplast. Melvin Calvin Laboratory, University of California, Berkeley; Steve Ruzin.

CBB 822-1649

Fig. 1."Energy"cane (left), Puerto Rico.

CBB 821-228

Fig. 2. Copaifera multijuga, Ducke Forest, Manaus, Brazil showing bung in trunk. (Ancona)

C. langsdorfii
Planted 12/8/80
Picture taken 8/5/81

C. officinalis
Planted 4/13/81
Picture taken 8/5/81

Baysdorfer

CBB 818-7824

Fig. 3. Copaifera seedlings, Melvin Calvin Laboratory, University of California, Berkeley.

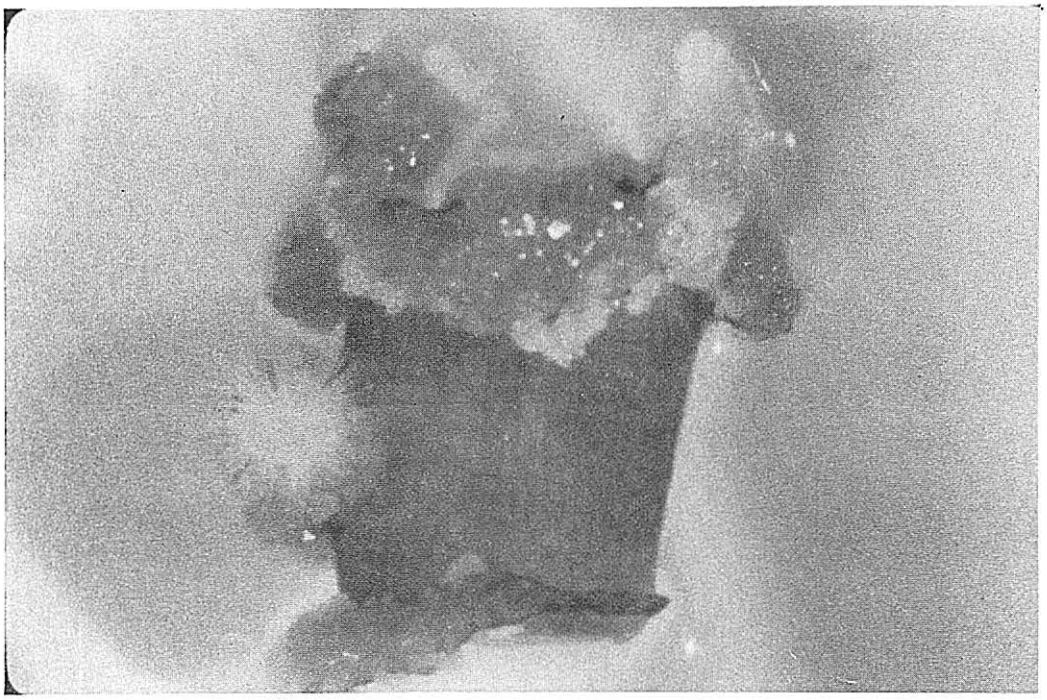

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Fig. 4. Euphorbia lathyris callus, Melvin Calvin Laboratory, University of California, Berkeley; K. Redenbaugh.

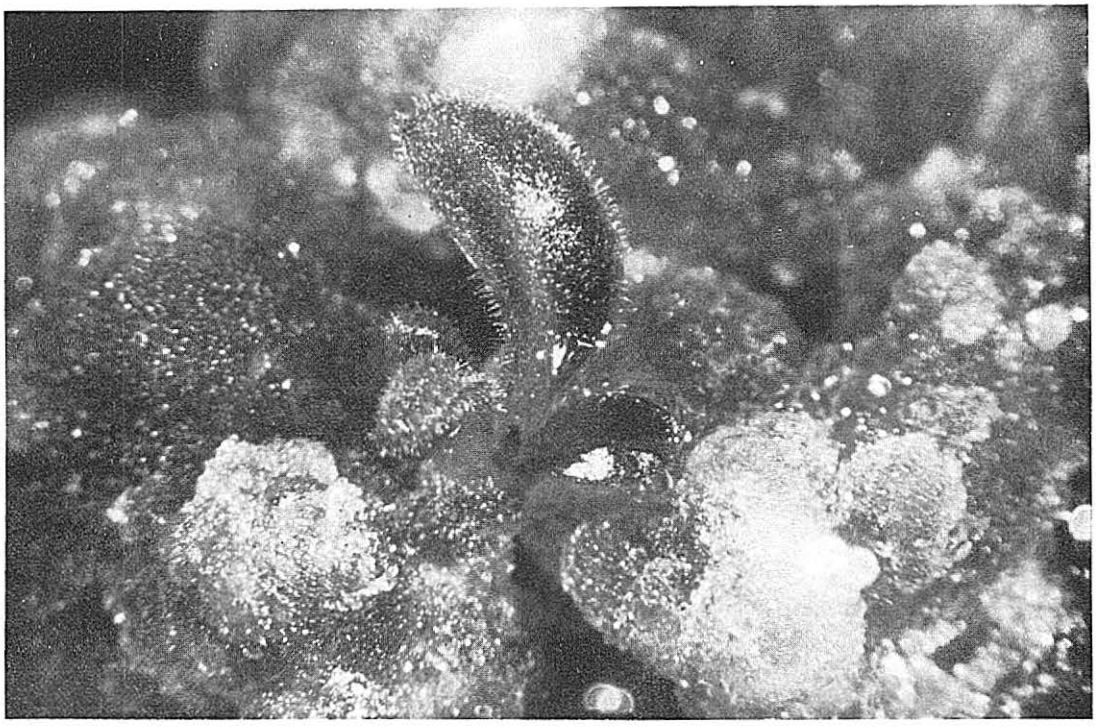

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Fig. 5. Euphorbia lathyris shoots from leaf protoplast. Melvin Calvin Laboratory, University of California, Berkeley; Steve Ruzin.

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