New Sources for Fuel and Materials

Melvin Calvin

Shortly after the 1973 oil embargo we began our efforts to find plants from which liquid fuel could be produced and used directly (*1*) as alternative energy sources. In Brazil, processes have been developed to use sugarcane to produce alcohol for use as a fuel additive; in 1974 the production of alcohol for use in gasohol was 400 million liters, and by 1981 it was 4.4 billion liters. In actuality, however, the alcohol produced from sugarcane in an enclosed system may be more useful as a chemical intermediate than as a fuel for automobiles.

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

In Puerto Rico, where sugarcane has been grown for carbohydrate, efforts have been made to improve the plant for higher cane production (*2*). This so-called "energy" cane (Fig. 1) produces about 250 tons of total biomass per hectare, but the sugar content per hectare is the same for both energy cane and ordinary sugarcane. The energy cane has three times as much product which can be used for such purposes as firing power boilers to produce electricity.

Isoprenoids from the Whole Plant

Most of the plants examined in detail so far belong to the family Euphorbiaeceae—the family to which the rubber tree (*Hevea brasiliensis*) belongs. Various species of *Euphorbia* grow in all types of climates throughout the world, but we were particularly interested in those which would grow on land not suitable for food production. The plant we studied in greatest detail is *Euphorbia lathyris*, which is grown on experimental plantations in both northern and southern California as well as on semiarid land near the University of Arizona (*3–6*). The processing sequence to recover fuels and materials from *E. lathyris* is simple in concept and related to that used to extract seeds and oils (*7*). The first step, when 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 that uses hexane), aqueous methanol is used to extract sugar, and the result is 200 tons of fermentable sugar. The bagasse that remains can be used to run the entire process—that is, 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 is to be the final product of the sugar. The material balance for 1000 dried tons of *E. lathyris* per day 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.

The oil obtained from *E. lathyris* after extraction (*8*) has been subjected to catalytic cracking with special zeolite catalysts developed by Mobil Oil Corporation (*9*). The usual suite of products results: ethylene (10 percent), propylene (10 percent), toluene (20 percent), xylenes (15 percent), C_5 through C_{20} nonaromatics (21 percent), coke (5 percent), C_1 through C_4 alkanes (about 10 percent), and fuel oil (about 10 percent). All these materials are useful for petrochemical industrial processes.

The main interest of this discussion, however, is in how the green plant makes these materials and whether there is anything we can do to "improve" the yield and quality of oil. The biosynthetic route by which the plant makes the oil is fairly well known; pyruvate is obtained from sugar through the glycolytic cycle, and is then built up to mevalonic acid and goes on to form isopentenylpyrophosphate, which is polymerized and forms a variety of isoprenoids. Normally, in the *E. lathyris* the material goes through the isoprenoid biosynthetic pathway to squalene (C_{30}) which is then folded up to make C_{30} terpenoid alcohols, which constitute the greater percentage of the oil.

Isoprenoids by Tapping the Tree

In another plant family in Brazil, the Leguminosae, there is a genus called *Copaifera* that contains trees which are prolific terpene producers. The product of the *Copaifera multijuga*, for example, is a sesquiterpene. This particular tree is harvested by drilling a hole in the trunk about 1 meter from the ground; the hole is about 2 centimeters in diameter and goes into the heartwood of the tree. A pipe is inserted into the hole, and the oil drains out of the pipe into a bucket. This operation can be done twice a year, and in 24 hours 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 heartwood, from pores (1 to 2 millimeters in diameter) that run vertically throughout the tree trunk. There are at least 25 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, in an effort to discover the mechanism of the formation of the diesel-like fuel in the trees and possibly to increase the yield of this material. Agronomic studies are under way as well to see whether *C. multijuga* could be established as a viable commercial crop. Also, it is not yet known whether more than one tap can be used in each tree.

The biosynthetic method by which the diesel-like oil from the *Copaifera* is made is the same as that of the *E. lathyris* up to

The author is University Professor of Chemistry and Faculty Senior Scientist of Lawrence Berkeley Laboratory, University of California, Berkeley 94720. He is the former director of the Laboratory of Chemical Biodynamics at Berkeley. This article is based on a paper presented at a symposium on Biomass Substitutes for Liquid Fuels in Campinas, Brazil, 9 to 12 February 1982.

0036-8075/83/0107-0024$01.00/0

the formation of C_{15}. In *Copaifera* the C_{15} farnesyl pyrophosphate is cyclized; that is. the phosphorus drops off and cyclic C_{15} compounds are obtained. One type of enzyme is responsible for the difference between the end products of *C. multijuga* and *E. lathyris*. In *E. lathyris* this enzyme, farnesyl pyrophosphate, is dimerized, whereas in *Copaifera* the material is cyclized with the result that many C_{15} products are formed. The oil from the *Copaifera* is used for medicinal purposes by Amazon natives, and it is a component in pharmaceutical products as well as being used directly as a fuel in automobiles.

Isoprenoids from Fruits and Seeds

There are a number of other plant species that have potentially useful oils. One, *Pittosporum resiniferum* (*10*), grows in the Philippines. The fruit of this plant is quite large and is used frequently for illumination: it is tied to the end of a stick and lighted. Analysis of the oil from these fruits (called petroleum nuts) indicates that the major products are α-pinene (38 percent), myrcene (40 percent), *n*-nonane (3 percent), and heptane (5 percent) (*11*). This fruit has terpenes, not glycerides, in it. However, another species that grows in California (*P. undulatum*) and has smaller fruits, gave slightly different products. The major ones were α-pinene and limonene.

Other potential sources of fuel and material are marmeleiro (Euphorbiaceae, Croton) from Brazil, andiroba (*Carapa guianensis*) also from Brazil, the copaiba sesquiterpenes (*C. multijuga*) from Brazil, monoterpenes from *Pittosporum undulatum* (California), and monoesters from jojoba. Four of these oils (from *E. lathyris*, *P. undulatum*, and copaiba) are terpenes, which have the desired characteristics for fuel and materials; the one from jojoba is a monoester, and that from 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 substitutes, particularly in farm machinery.

Gene Transfer

We would like to transfer the gene for the production of sesquiterpenes from *C. multijuga* (or other *Copaifera* species) to such plants as *E. lathyris* because it is not possible to grow *Copaifera* in the United States. Gene transfer would be one way to use the characteristics of *Copaifera* in a species that does grow here. As mentioned earlier, there is only a single enzyme, farnesyl pyrophosphate cyclase, involved in the cyclizing of the C_{15} pyrophosphate. In other words, a single gene transplant from the donor cell of the *Copaifera* to the acceptor cell of *E. lathyris* (*12*) would be required. It is necessary, however, to find a donor cell which has the genes for the appropriate enzyme, get the messenger out, make a copy of the DNA, insert the copy DNA into a plasmid, clone the plasmid in

Fig. 1. Energy cane (left), Puerto Rico. [Photograph by G. E. Calvin]

Fig. 2. *Copaifera* seedlings, Melvin Calvin Laboratory.

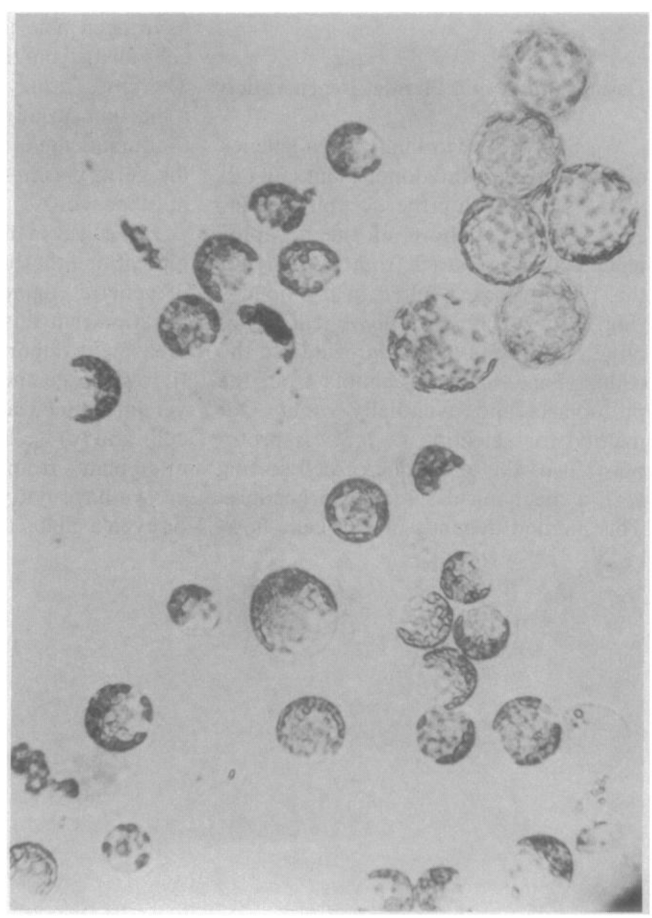

Fig. 3. *Euphorbia lathyris* mesophyll protoplasts. [Photograph by K. Redenbaugh]

Fig. 4. *Euphorbia lathyris* shoots from leaf protoplast. [Photograph by S. Ruzin]

Escherichia coli and then, by means of the plasmids, insert the gene into a selected plant such as *E. 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.

Tissue Culture and Plantlet Regeneration

We have been working with a species of *Copaifera* for the donor plant (Fig. 2) and *E. lathyris* for the acceptor plant. First a tissue culture of the acceptor plant cell is prepared from *E. lathyris*; this process has resulted in protoplasts (Fig. 3) (*13*). The *E. lathyris* leaf mesophyll protoplasts have aggregated to the callus (Fig. 4), getting shoots from the protoplasts and eventually roots. Our method for selecting *E. lathyris* protoplasts uses the technique of cell sorting (*14*), a mechanical selection procedure. This method depends on the cells flowing past a laser beam with a number of light detectors. One cell is stained with fluorescein, which fluoresces yellow, and the other is stained with rhodamine, which fluoresces red. Cell fusion would lead to a cell that fluoresces in two colors, and it should 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 may now be possible to use a mechanism of genetic manipulation at the somatic level without interfering with the germ plasm—somatic hybridization, in other words.

There are many uncertainties in manipulations that use the new technology of genetic engineering, but there is no question that this technology will be increasingly important. We have learned (i) to separate and fuse cells but have not yet introduced any new genes into the cells and (ii) 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 known, 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 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 indicate the feasibility of using hydrocarbon-producing plants for energy agriculture. The next steps will be to refine tissue culture and plant cloning techniques so that suitable enzymes from one plant can be introduced into another to produce the chemicals most useful for fuels and materials and, finally, to build the pilot plants to extract and process the oil for use.

References and Notes

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15. Supported in part by the Office of Renewable Energy, Biomass Energy Technologies Division of the Department of Energy under contract DE-AC03-76FS00098.