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FUEL OILS FROM EUPHORBS AND OTHER HIGHER PLANTS

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Presented at the International Symposium on Chemistry,
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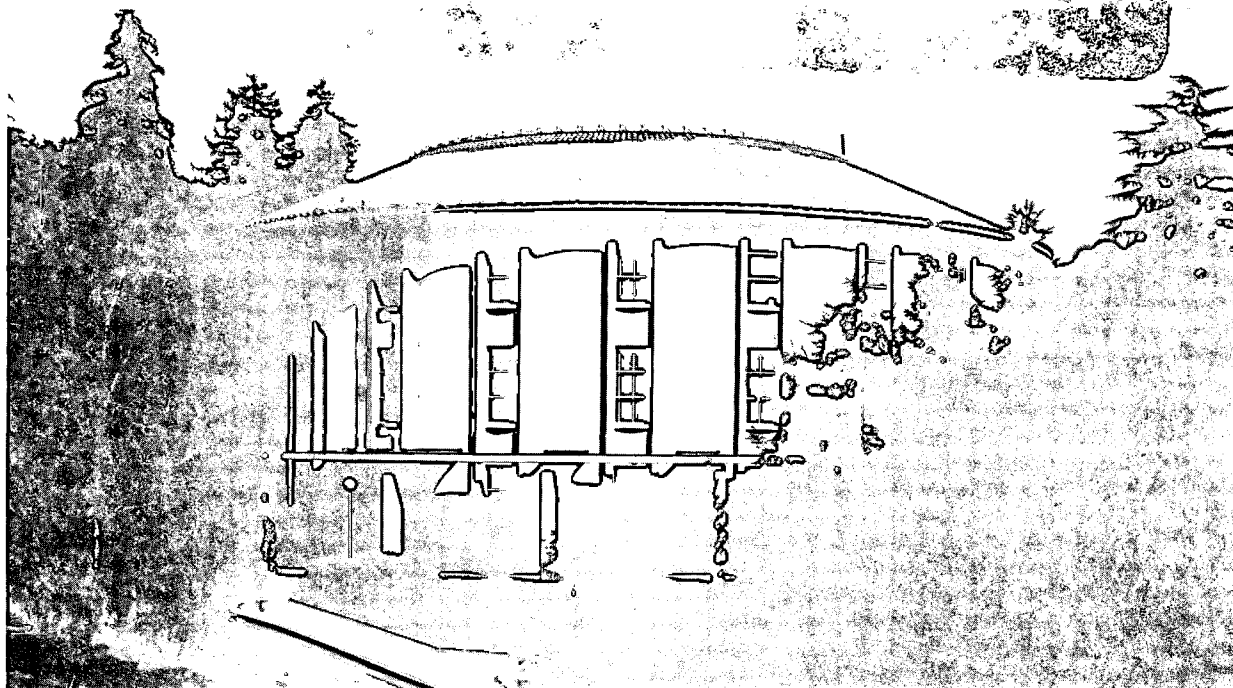
FUEL OILS FROM EUPHORBS AND OTHER HIGHER PLANTS

M. Calvin

May 1986

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FUEL OILS FROM EUPHORBS AND OTHER HIGHER PLANTS

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ABSTRACT

The increasing energy costs of finding petroleum, together with the sure knowledge that its supply is finite, has prompted us to seek other sources of liquid hydrocarbon for both fuel and material. We have turned to annually renewable plant sources such as seed oils, an obvious source, with palm oil as the most productive. Sugar cane to ethanol is a second source already in use.

We have examined non-food plants which can be grown on marginal soil for their productivity, particularly the genus *Euphorbia*, all species of which produce a latex which can be converted into useful fuel and material as well as the raw material for what might be a valuable anti-tumor agent. *Euphorbias* and other similar plants, however, require repeated planting and harvesting of the entire plant, which constitute a drain on the soil. Trees can be longterm sources for hydrocarbon-like materials with a single planting. Examples are: The genus *Copaifera* which can be tapped for sesquiterpenes, the genus *Pittosporum* which bears fruits rich in terpenes and can be harvested annually. Finally, there are algae whose oil productivity is already of interest.

It seems possible to modify genetically the terpene biosynthetic pathways in plants to improve both the quality and quantity of the oils produced from them.

With the oil embargo of the early 70's we were forced to concern ourselves with matters of fuel resources. (Calvin, 1976, 1977, 1979, 1980, 1982, 1983a, 1984; Hoffman, 1983; Lipinsky, 1981, McLaughlin et al. 1983) The price of oil today (1986) is an aberration which also will not last very long and should not divert us from developing domestic sources along renewable avenues.

The first thing suggested by many of our economists is that we should turn our attention to using coal in a more efficient and environmentally satisfactory manner. There is an environmental consequence of the use of any fossil fuel, but especially coal, which cannot be eliminated: That is the fact that when fossil carbon is burned enormous amounts of carbon dioxide are produced. Even within the last 50 years when most of our fossil combustion has been not carbon (coal) but hydrogen (oil/gas) the CO₂ level in the atmosphere has risen. This is a consequence which would accelerate if coal was used as a major fuel source, either indirectly (in the form of liquid fuel) or directly. Burning coal produces approximately twice as much CO₂ per unit energy as hydrocarbon, where both carbon and hydrogen is burned.

In the last few years we have been dominated by hydrocarbon rather than coal, and even under those circumstances the rate of production of CO₂ in the atmosphere has been roughly twice as great as the rate at which it is being removed either by both the oceans and the biosphere. (Sundquist & Broecker, 1985; MacCracken & Luther, 1985). This global trend is illustrated in Figure 1 which shows the variation of CO₂ concentration in the atmosphere during the past geological time scale as well as a modern human one. One hundred years ago the CO₂ concentration in the atmosphere was approximately 290 ppm and the concentration today is 315 ppm. The expectation is that the CO₂ concentration will continue to rise.

It is possible to detect warming consequences of rising CO₂ concentration by using a device which integrates the temperature fluctuation over a long period of time. There are two ways this can be done, one by examining the size of the polar icecaps and glaciers from satellite pictures and also from surface measurements. These indicate that the Antarctic icecap has decreased substantially in the last 100 years. If substantial quantities of ice have melted from the polar icecaps and glaciers, there should be a rise in sea level (Figure 2) which would thus be related to increased combustion of fossil fuel. The evidence is available that the earth's temperature is increasing and the consequences of that increase are measurable. As a result of the increased temperature and loss of coastal areas due to higher ocean levels there will be very severe worldwide consequences for agriculture, the agricultural pattern across the surface of the earth will change markedly. For example, the plains of the United States might no longer be capable of growing grain; Northern Canada and the Soviet Union would become the chief sources of grain for the world. It will be very difficult for the human race to adjust such an enormous change in agricultural patterns in the 20-30 years which will elapse for this rise in sea level to manifest itself catastrophically.

HYDROCARBON-PRODUCING PLANTS

We must look for our liquid fuel (hydrocarbons) in the form of a renewable resource that can be grown each year. There is one plant whose cultivation for the purpose of renewable fuel and materials is already underway, i.e., the sugar cane in Brazil. (Geller, 1985) In 1975 the Brazilians produced 700 million liters of ethanol from sugar cane and in 1985 the production was 7 billion liters. The Brazilians are producing more than 20% of their total liquid energy needs from alcohol and they are beginning to create a chemical industry--the sucrochemical industry--based on this energy source.

It seemed worthwhile to look for plants which would carry out the reduction of carbon dioxide all the way to hydrocarbon instead of half way to carbohydrate. (Calvin, 1977, 1979, 1980, 1983, 1984, 1985; Lipinsky, 1981; McLaughlin et al. 1983; Nemethy, 1984) This process is exemplified in the seed oils which have substantial possibilities. The oil content and average oil yield for some oil crops is shown in Table 1, with palm oil the most productive. Peanuts, safflower and sunflower are the most important seed oils in the United States and all of them produce triglycerides. The oil content of the seeds represents a reasonable opportunity for renewable fuels. (Amer. Soc. Agric. Engineers, 1982; Shultz & Morgan, 1984; Princen, 1983; Harington, 1986) Sunflower cultivation has been expanded in the United States as a real possibility to provide diesel fuel for farm machinery. The oil itself, a triglyceride (glycerine with three fatty acid chains attached to it) as it comes from the seed is not a very satisfactory diesel fuel, but by treating it with methanol the fatty acids can be transesterified so the byproduct is free glycerine and the methyl esters of the fatty acids. This latter material can be used directly as diesel fuel.

There are also plants which take the initially produced carbohydrate and instead of converting it into fatty acids and glycerides (such as the seed oils) convert it into terpenes. The most important commercial plant of this type is Hevea brasiliensis, a member of the Euphorbiaceae family which makes polyisoprene rubber. Other members of the Euphorbiaceae family produce hydrocarbons, especially the genus Euphorbia which has 2000 species of all sizes which grow throughout the world. Every Euphorbia species contains a latex which is an emulsion of about 30% terpenes in water. The latex hydrocarbon is largely a C₃₀ triterpenoid which can be cracked like oil to make high octane gasoline. (Weisz and Marshall 1979) The zeolite catalyst cracking of the crude oil from the E. lathyris resulted in the usual group of products, similar to those obtained from standard cracking of petroleum, such as olefins, paraffin, aromatics and nonaromatics. This information confirms the desirability of the products of E. lathyris as possible raw materials to substitute for crude oil.

We started experimental cultivation of Euphorbias in California about ten years ago and Euphorbia lathyris (Figure 3) was the first hydrocarbon producing plant studied to test the hypothesis that plants could be grown for fuel and chemical content in marginally suitable land. (Calvin, 1977, 1979; Nielsen et al. 1977) The entire E. lathyris

Table 1

Oil Content and Average Oil Yield for Some Oil Crops

Oil Crop (Location)	Oil Content wt. %	Average Oil Yield kg/ha
Palm oil (Malaysia)	20	3,475
Copra (Philippines)	65-68	800
Peanuts (U.S.)	45-50	790
Safflower (U.S.)	30-35	762
Sunflower (U.S.)	40-45	589
Rapeseed (Canada)	40-45	409
Soybean (U.S.)	18-19	319
Corn kernel (U.S.)	4-8	254
Flaxseed (U.S.)	35-42	230
Sesame (India)	45-50	220
Cottonseed (U.S.)	18-20	140

HYDROCARBON-PRODUCING TREES

One problem with using annual herbaceous plants as sources of hydrocarbons is soil erosion. It seemed better agronomic practice to grow trees (Seiberg, Folger & Milner, 1986) and either harvest the fruit or tap the tree for oil.

A tree that seems to be a likely candidate is Pittosorbum resiniferum (Figure 7) a member of the Pittosporaceae family which grows in The Philippines. The fruit of this particular tree is rich in light oil, containing about 30% terpenes. The fruits can be picked and distilled and thus the terpene-like fuel extracted. The composition of the steam-distilled oil from P. resiniferum fruit contains roughly four separate components (Figure 8), the most predominant being α -pinene and β -pinene, both C_{10} compounds. (Nemethy & Calvin 1982) The results indicate that the fuel properties of steam distilled oil from P. resiniferum fruits after hydrogenation are quite comparable to those of gasoline.

The Copaifera multijuga (a member of the Leguminosae family) grows in the Amazon region of Brazil and produces C_{15} hydrocarbons. The oil is light yellow, very similar in appearance to olive oil, and is obtained by tapping. (Alencar, 1982) A hole is bored horizontally in the trunk of the C. multijuga tree into the heartwood (Figure 9) and a bung is placed in the hole. The bung is removed at certain times of the year and the oil flows directly into a container. A single hole in a large tree may yield about 25 liters of oil in 24 hours. The bung is reinserted and 6 months to a year later another 25 liters of oil is drained out.

The main components of the Copaiba oil, as it is commonly called, are caryophyllene, bergatomenene and copaene, all cyclic C_{15} compounds. This oil has also been subject to the Mobil zeolite catalyst process, as was the oil from E. lathyris, and the results indicate that it can be cracked into a useful suite of compounds. The characteristic that makes this species so attractive is the fact that the material from the tree can be used directly in a diesel engine without any further processing.

OIL PRODUCTION BY ALGAE

There is another candidate for oil production which grows in many parts of the world where it occurs primarily in fresh waters. This is the unicellular green alga Botryococcus braunii which produces terpenoid oils. (Wolf, 1983; Wolf et al. 1985). Colonies are often observed floating on the surface of undisturbed waters and this buoyancy is due to the large amounts of accumulated oil in the alga. The hexane-extracted Botryococcus oil is orange as a result of the presence of carotenoids. After the removal of the pigments a clear oil is obtained that contains a homologous series of unusual isoprenoids. The structure of the C_{34} component (Botryococcene) (Cox et al. 1973) has been determined and more recently the structure of the C_{36} compound (Darwinene) (Galbraigh, Hillen & Wake, 1983) has also been elucidated (Figure 10). This alga is a very real candidate. We have learned how to

plant is harvested, and from the harvest 8% of the dry weight is extracted as terpenes (oil), 20% of the dry weight as fermentable sugars, which leaves a residue of about 65% as lignocellulose. The terpenes can be cracked like crude oil and the sugar can be fermented like sucrose, while the lignocellulose can be used in a way similar to the bagasse of sugar cane. The products of the extraction of E. lathyris represent a new possibility for a future energy and materials source. The conceptual processing sequence to recover terpenoids and sugars is shown in Figure 4. (Nemethy, Otvos & Calvin, 1979, 1981) The oil from the E. lathyris is black and tarry and resembles crude oil and consists mostly of triterpenes which are steroids and steroid esters (C_{30} compounds).

Some of the latex sterols of the Euphorbia lathyris latex (Figure 5) are important in the pharmaceutical industry and could conceivably be of more value than the actual crude oil obtained. A very small percentage of the latex hydrocarbons consist of C_{20} components, some of which are related to ingenol (Figure 6). Certain ingenol esters are potent stimulants for cell division and also create an irritant property which is harmful to the skin, mucous membranes of the nose, eyes, etc. (Adolf & Hecker, 1975; Furstenberger & Hecker, 1985; Bissell et al. 1981) If the ingenol is converted into the dibenzoate ester it becomes an anti-tumor agent. (Kupchan et al. 1976). There is a definite relationship between the two biological effects. The stimulant has the long chain on it which stimulates cell division. The binding sites are presumably the same for the dibenzoatedibenzoate probably determined by the structure of the C_{20} skeleton. For the dibenzoates of the basic structure of ingenol there is no surface active component and presumably the material binds to the binding site and reverses the stimulation of cell growth and thus has an effect on leukemic mice.

BIOSYNTHETIC PATHWAYS

It might be possible to modify the products of the Euphorbias by modifying their biosynthetic routes to triterpenes. The terpenes in hydrocarbon-producing plants and trees are probably made by a well-known biosynthetic pathway: sugar to pyruvic acid to mevalonic acid to isopentenylpyrophosphate (IPP). A portion of the unsaturated IPP is isomerized to dimethylallylpyrophosphate (DMAP), and the two isomers are combined. The allylic phosphate comes off DMAP and the resulting carbonium ion attacks the double bond of IPP, followed by proton loss, which results in exactly the same allylic structure as before. Eventually, if the process continues, rubber is the result.

A comparison of biosynthetic routes might be useful. In the case of the Euphorbia lathyris the sequence produces C_{15} compounds which dimerize to C_{30} followed by cyclization to produce triterpene steroids. In Pittosporum, however, the route is to the C_{10} compounds at which cyclization occurs to create monoterpenes in the fruit. The biosynthetic method by which the "diesel" oil from the Copaifera is made is the same as that used by the E. lathyris up to the C_{15} step. Copaifera cyclizes the C_{15} farnesyl pyrophosphate, producing cyclic C_{15} compounds.

GENETIC ENGINEERING

It would be very useful to be able to obtain from the Euphorbias a product similar to the Copaiba oil. (Calvin *et al.* 1982) To achieve this it seems that a single gene could be transferred from the plant that cyclizes the C₁₅ from farnesyl pyrophosphate into a plant such as the Euphorbias which has all the other enzymes already but which goes on to create C₃₀ materials. It appears that only one class of enzyme, the farnesyl pyrophosphate cyclase, is needed for this purpose. In other words, a single gene transplant from a donor cell of the Copaifera to the acceptor cell of a Euphorbia would be required. To perform this operation it would first be necessary to extract the required gene from the plant(s) which has it and insert it into a plant that does not have it. One relevant possibility would be to take the protoplasts from the two plants (Copaifera and Euphorbia) and have a fusion/selection procedure for plant regeneration (Figure 11) (Redenbaugh, personal communication; Calvin, 1983b) We have been able to reproduce a Euphorbia plant from a protoplast. A single protoplast in culture aggregates very quickly into a callus; the callus is adjusted with various hormones to form shoots and roots and thus regenerates a cloned plantlet.

CONCLUSION

A summary of energy yields from different hydrocarbon producing plants is shown in Table 2 in terms of the yield of liquid fuel/acre/year/inch of water. This indicates the work is underway with different plants of different types to develop the idea of using biomass as a significant component of total energy use, particularly in the United States. It has been predicted that by the year 2000 biomass will represent approximately 6% of the total energy used in the United States. The idea of using plants to create hydrocarbon-like materials as a substitute for our current fuel and materials sources will become more important, especially in some of the less developed areas of the world which have a great deal of land not suitable for food production. (Calvin, 1985) Various efforts are being made toward this end in Okinawa, Thailand, Australia, and Spain, and attempts are underway to improve agronomic yields, develop small scale extraction plants, learn more about the composition of the plant oils and study possible ways of modifying the biosynthetic routes (particularly with cyanobacteria) to produce more desirable end products.

The idea of growing hydrocarbons is not new. It was first promulgated by the Italians in 1936 in Ethiopia. (Frick, 1938) They were running out of oil there and thought they would grow plants such as Euphorbia Abyssinica, extracting the oil from these plants and using it for their vehicles. This development was not pursued because of the war. Similarly, the French in Morocco in 1940 developed plantations for growing Euphorbia resiniferum over a period of several years; however, however, this also was abandoned because of World War II. (deSteinheil, 1940)

Table 2
Comparison of Energy Yields for Different Crops

Process	Dry Biomass Yield, Tons Acre ⁻¹ Yr	Liq. Fuel Yield/Acre Yr ⁻¹	Water Req. In. Yr ⁻¹	Energy in Liq. Fuel (10 ⁸ Btu) Acre ⁻¹ Yr ⁻¹ per Inch of Water	Cellulosic Residue Acre ⁻¹ Yr	Energy in Cellulose (10 ⁶ Btu) per Acre Yr ⁻¹ per Inch
Corn to ethanol	5	16x10 ⁶ Btu (0.64 tons)	25	0.65	44.2x10 ⁶ Btu (3.4 tons)	1.77
Sugar cane to ethanol	30	60x10 ⁶ Btu (2.4 tons)	78	0.78	312x10 ⁶ Btu (24 tons)	4
Energy cane to ethanol	35-50	65x10 ⁶ Btu (2.56 tons)	48	0.35	400x10 ⁶ Btu (31 tons)	8.2
<u>Euphorbia lathyris</u> to hydrocarbons and ethanol	8.5	20x10 ⁶ Btu (0.58 tons) 17.3x10 ⁶ Btu (0.68 tons)	25	0.82	79.6x10 ⁶ Btu (6.12 tons)	3.2
<u>Pittosporum</u> <u>resiniferum</u> (fruits only) to hydrocarbons	7.8	50x10 ⁶ Btu (1.5 tons)	~25	2.0	101x10 ⁶ Btu (7.8 tons)	4.0
<u>Jatropha curcas</u> (seed only) to hydrocarbons	5.0	92x10 ⁶ Btu (2.2 tons)	~25	3.6	36x10 ⁶ Btu (2.8 tons)	1.45
Palm (fruit)	8.1	73x10 ⁶ Btu	(~25)	(2.9)	(~1 ton)	

What is needed now is an effort on the part of the agricultural and energy community to commit itself to an energy agriculture which would have long term benefits for the entire world.

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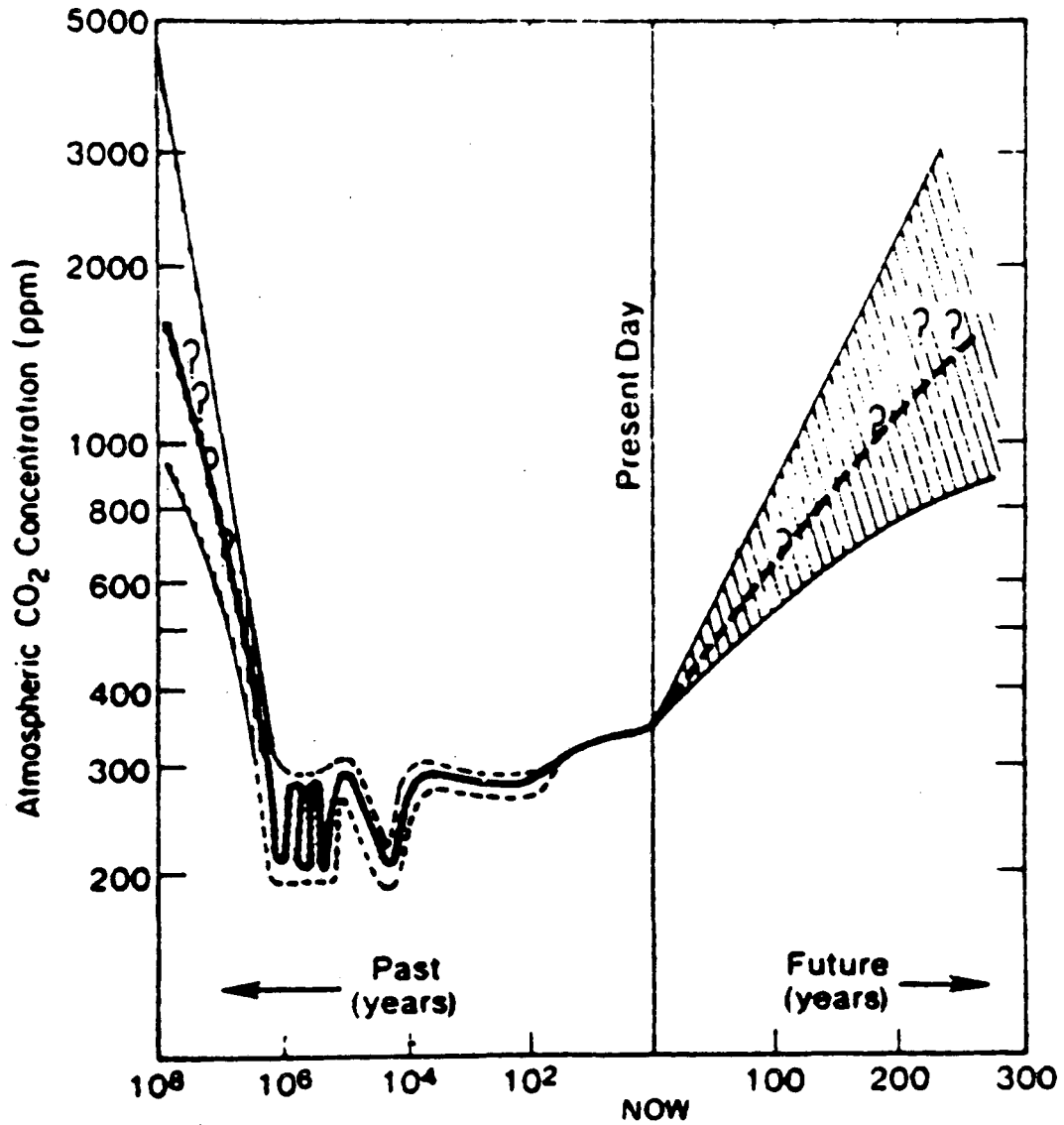
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FIGURE CAPTIONS

- Figure 1 Concentration of CO₂ in earth's atmosphere, past and future
- Figure 2 Global mean sea level trend based on tide gauge measurements
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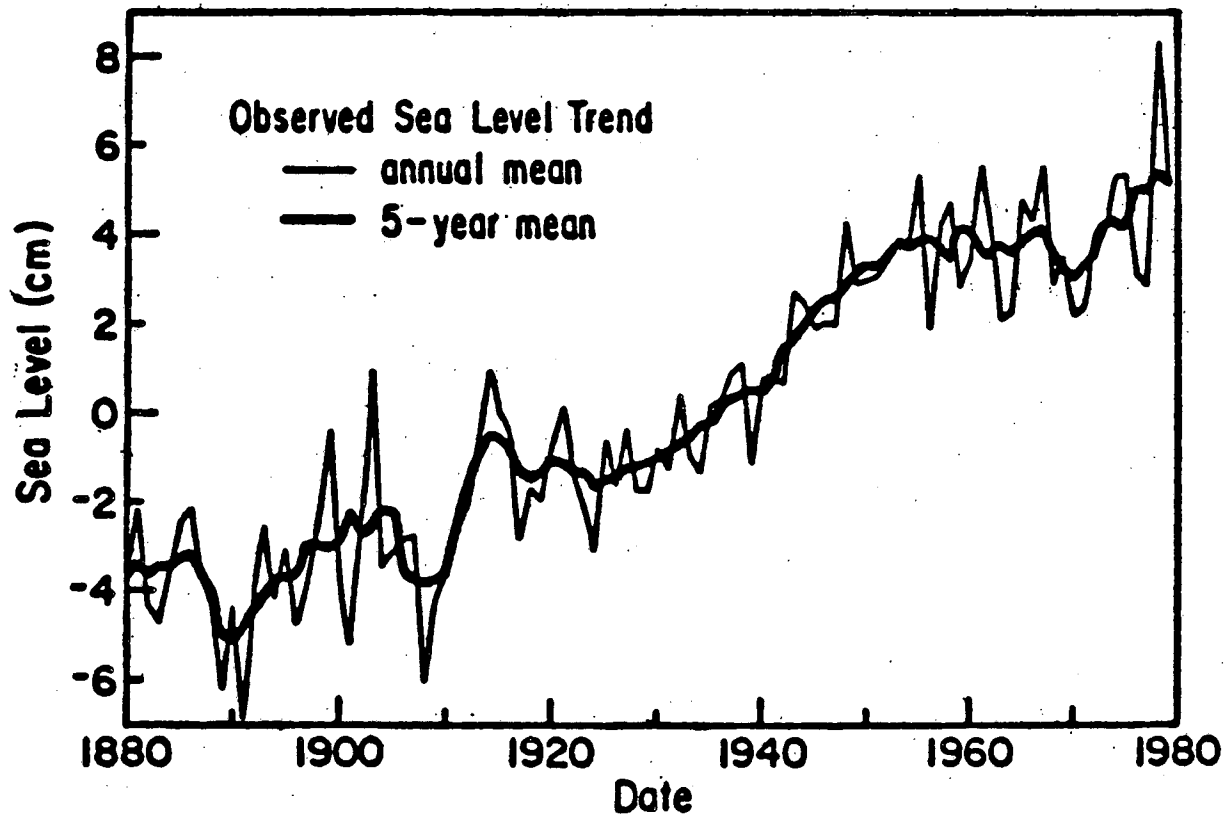
CARBON DIOXIDE: PAST AND FUTURE



(Gammon, Sundquist & Fraser)

XBL 865-1868

Fig. 1



Global mean sea level trend based on tide gauge measurements.

XBL 825-10138

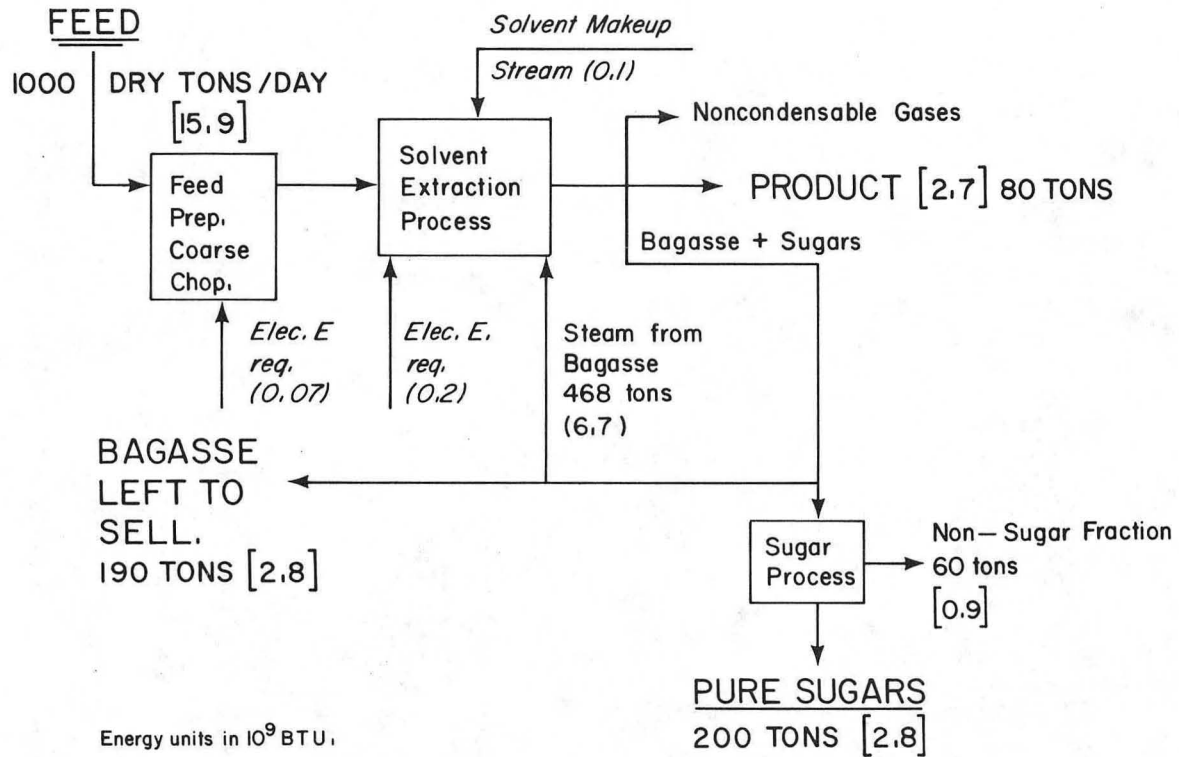
Fig. 2



Fig. 3

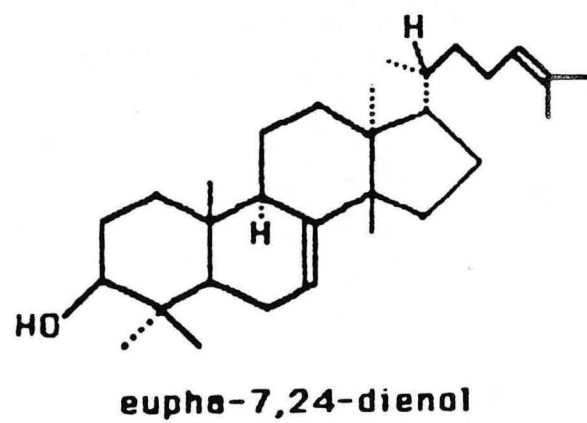
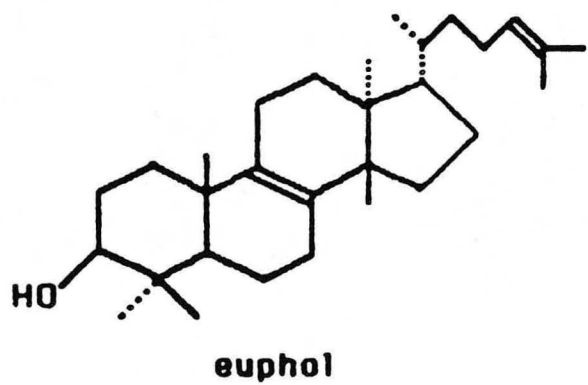
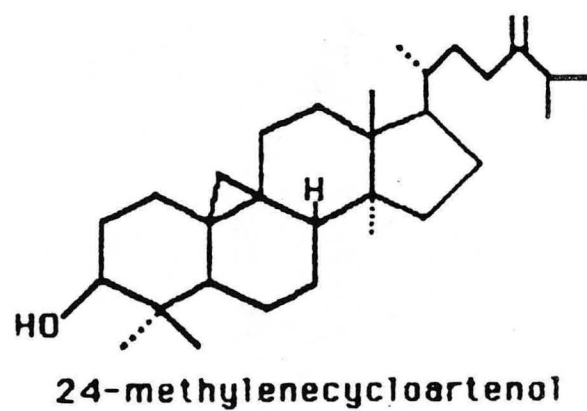
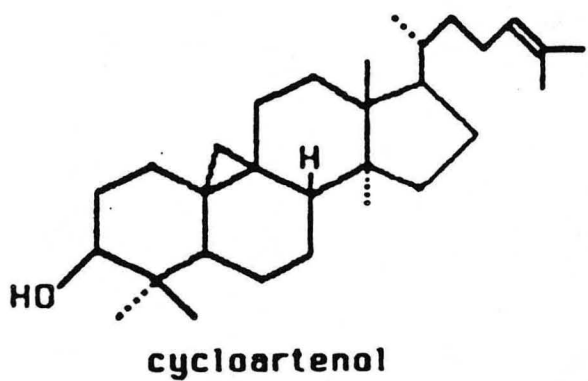
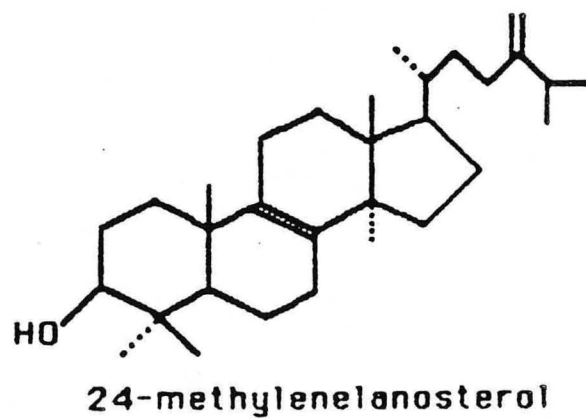
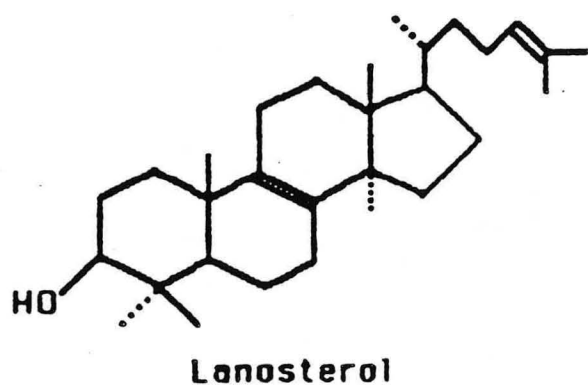
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Conceptual Processing Sequence to Recover Terpenoids and Sugars from Euphorbia lathyris



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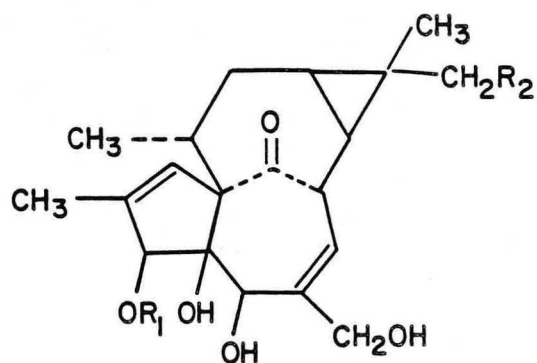
Fig. 4



THE LATEX STEROLS OF EUPHORBIA LATHYRIS

Fig. 5

Found in E. Lathyris



Active Esters of Ingenol:

R_1 = hexadecanoate

R_2 = H

R_1 = 3-tetradeca-2,4,6,8,10-pentaenoate

R_2 = H

Inactive:

16-hydroxy ingenol: R_2 = OH

W. Adolf and E. Hecker Z. Krebsforsch, 1975

Fig. 6

XBL 815-4637



Fig. 7

XBB 852-1110

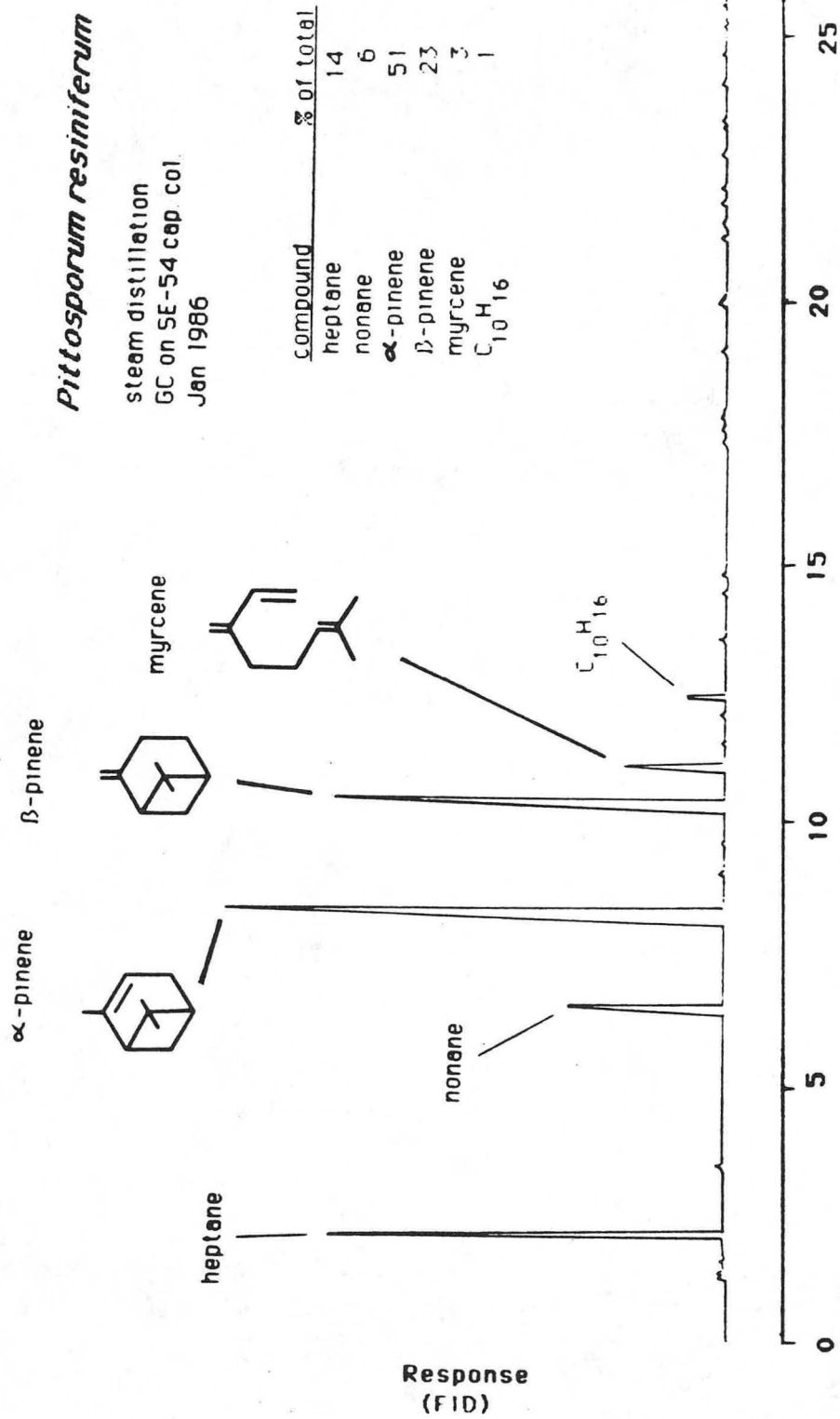


Fig. 8

XBL 863-863

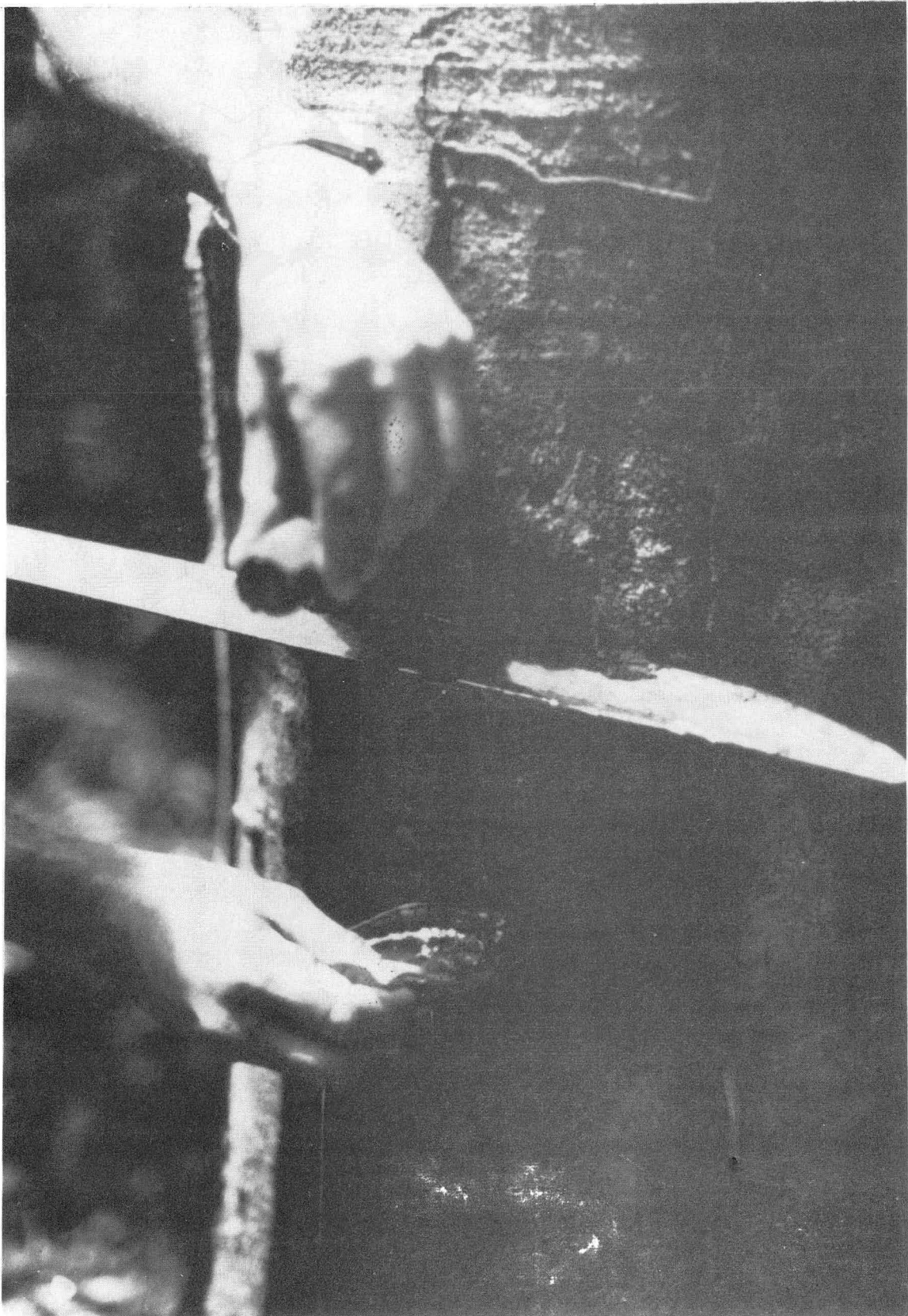
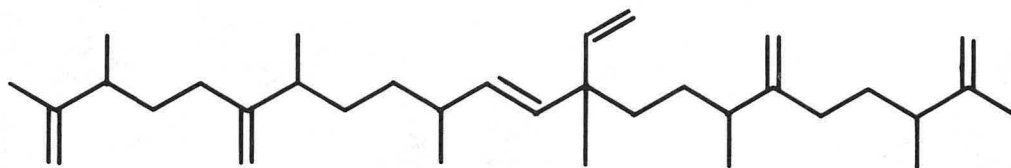
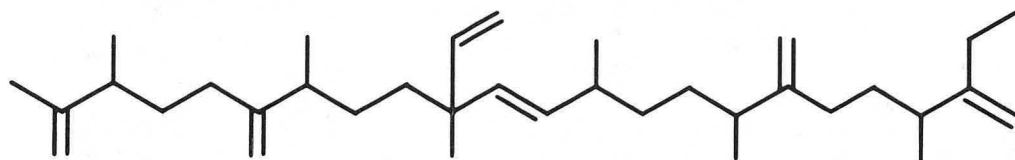


Fig. 9

CBB 821-230



botryococcene



darwinene

XBL8310-600

Fig. 10

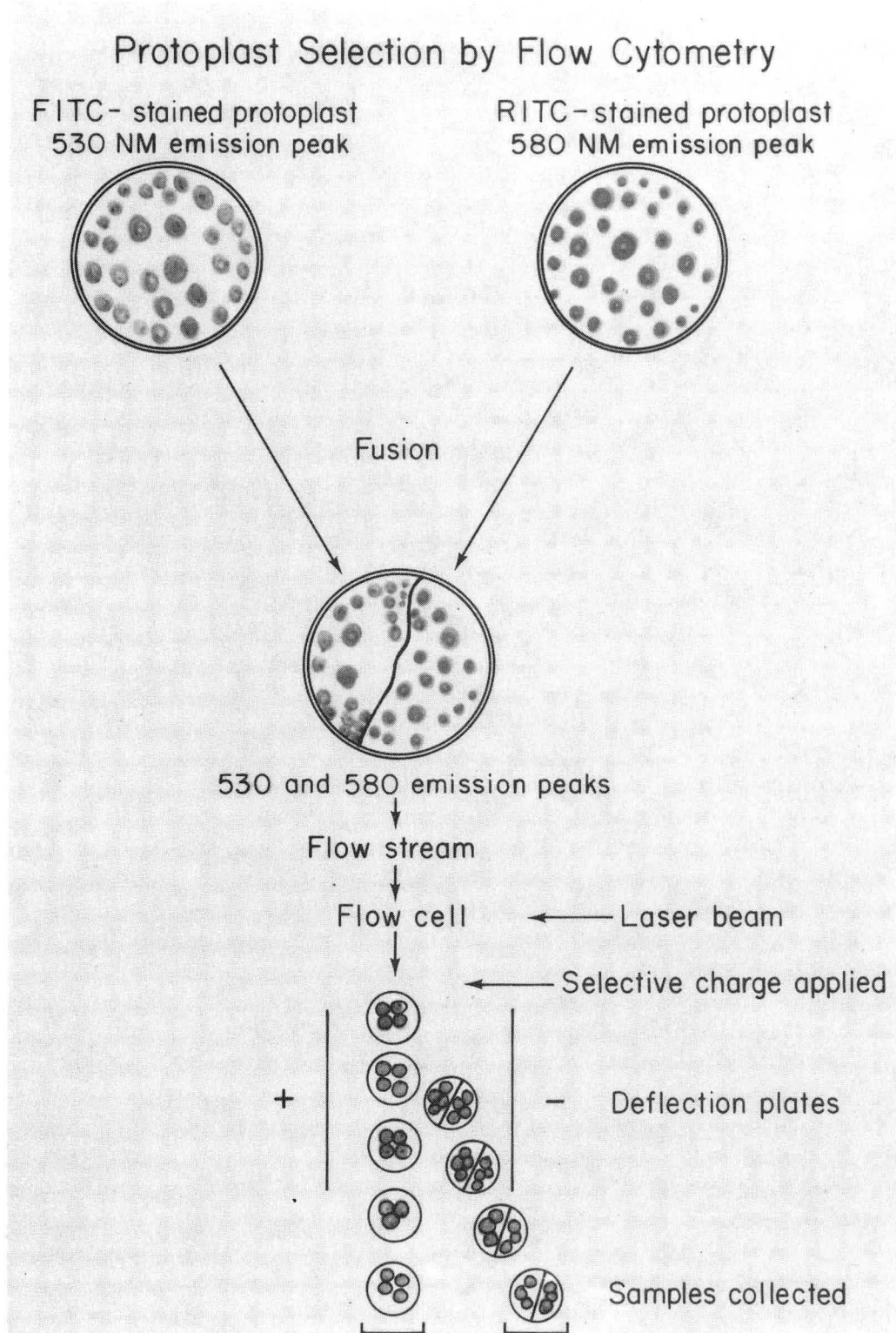


Fig. 11

CBB 815-4588

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