absent, and that the *H-Y* locus in this case must have been on the long arm of the Y chromosome. This location, if near the centromere, is compatible with the results obtained in most of the other cases studied, with the exception of case 5 and (probably) case 7.

These discrepancies need to be reconciled. Perhaps a pericentric inversion of the Y chromosome altered the position of the locus in either cases 5 and 7 or in case 8. The Y chromosome in man, as in most animals, does not show the same type of meiotic pairing with the X, involving chiasma formation and crossing-over, as do the autosomes with their homologs (*12*). Therefore, inversions involving the Y chromosome need have little, if any, effect on fertility unless a breakpoint falls within the *H-Y* locus, and a population of individuals could contain a variety of Y chromosomes with the position of the *H-Y* locus different in each one. In this regard, it is interesting that in each of our cases with an abnormal Y chromosome the findings indicate a locus relatively close to the centromere despite discrepancies regarding which arm it is on.

Our finding can be explained by a simple mechanism: the existence of two regions highly susceptible to breakage, one in each arm of the Y chromosome, distal to the *H-Y* locus. Rejoining of the broken ends could produce either an inversion or a ring chromosome. The inversion would have no phenotypic effect unless there was a position effect due to transfer of a gene from a euchromatic region to a heterochromatic one (*13*). Whether the *H-Y* gene is more commonly located in the long arm or the short arm can be determined only by further study.

We have proposed that H-Y antigen is the product of the primary male-determining gene in mammals (*14*). According to this hypothesis, male differentiation of the initially indifferent embryonic gonad occurs in the presence of H-Y antigen and female differentiation occurs in its absence. Localization of the *H-Y* gene to a region on the short arm of the human Y is consistent with this hypothesis because the male determining gene or genes has been assigned to this region on the basis of findings in a number of cases (*15*). However, some cases are known in which a long arm location of male determining genes seems likely. For example, Siebers *et al.* (*16*) found a female phenotype and streak gonads in a patient with a presumptive 45,X,i(Yp) karyotype. The minute metacentric chromosome was said to have identical banding on the two arms and was thus interpreted as an isochromosome of the short arm of the Y. There is insufficient evidence at this time to rule out a long arm location of the *H-Y* locus in some individuals or even to hazard a guess at the proportion of human Y chromosomes with an *H-Y* gene at a given location.

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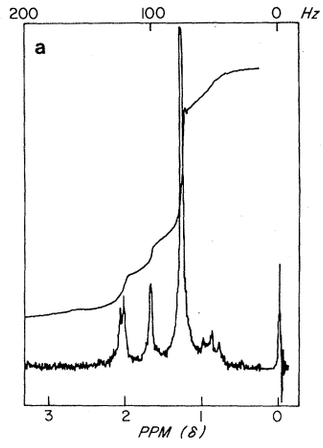
Plant Crops as a Source of Fuel and Hydrocarbon-Like Materials

Abstract. *Chemical analyses have been made of a number of plant species in order to assess their suitability as renewable sources of hydrocarbon-like photosynthetic products. Yields of rubber and wax, glycerides, isoprenoids, and other terpenoids were estimated. Individual sterols were identified in latex from some species.*

It has been suggested (*1*) that certain plants rich in polyisophrenes and other hydrocarbon-like materials might be cultivated and grown as renewable sources of highly reduced photosynthetic products. Two distinctly different agricultural methods can be applied in approaching this problem. Either we can harvest whole plants as suggested in a biomass plantation (*2*) or we can tap latex-containing plants as is done in the production of natural rubber (*Hevea brasiliensis*).

In order to evaluate the prospects of this idea, we have started a program of chemical analysis of both whole plant extracts (Table 1) and of plant latex (Table 2) (*3*). As can be seen from Table 1, as

Fig. 1 (top right). (a) The NMR spectrum of the benzene extract from *Euphorbia tirucalli*. The resonance at 1.2 parts per million (ppm) is assigned to $-(CH_2)_n-$ (wax) and the resonances at 1.6 and 2.0 to 2.1 ppm are assigned to methyl and methylene groups, respectively, in *cis*-polyisoprenes (rubber). (b) The NMR spectrum of acetone extract from *Jatropha curcas*. The resonance at 1.2 ppm is assigned to $-(CH_2)_n-$ (glycerides) and resonances at 1.5 to 1.6 ppm and 1.9 and 2.0 ppm were assigned to methyl (*cis* and *trans*) and methylene groups in polyisoprenes. Fig. 2 (bottom right). Gas-liquid chromatography (GLC) traces of acetylated sterols isolated from latex. The GLC separation was done on 3 percent OV-17 or 3 percent Dexsil 300 at 280°C (isothermal) and the identification of the sterols was made by coinjections with standard compounds and by comparison of their mass spectra (GC-MS, 70 ev) with those of standard compounds.



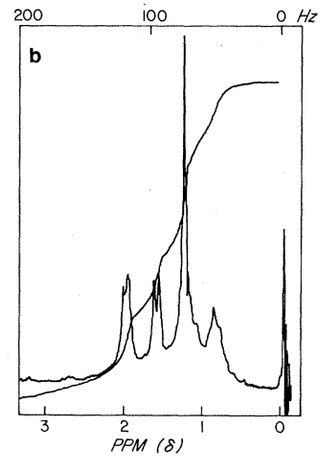


Table 1. Acetone and benzene extractables from various plants. Data are given in percent of plant dry weight. The plants (obtained from the collection of the Botany Department at the University of California, Davis) were dried in air, finely ground in a mortar, and extracted in a Soxhlet apparatus first for 8 hours with acetone and then for 8 hours with benzene. The solvents were evaporated, and the residue was taken up in $CDCl_3$. The compound distributions were estimated on the basis of 60-Mhz nuclear magnetic resonance spectra (NMR) (Fig. 1).

Plant	Benzene extract			Acetone extract			
	Rubber	Wax	Total	Total	Glycerides	Isoprenoids	Other (terpenoids)
Asclepias cursavica	0.6	<0.1	0.7	5.9	3.0	<0.5	2.0
Cryptostegia grandiflora	0.2	0.05	0.35	13.3	7.0	<0.5	6.0
Eucalyptus globulus	<0.01	0.05	0.1	12.0	3.5	<0.5	7.0
Euphorbia lathyris (leaves)	0.1	0.2	0.3	25.0	13.7	2.2	8.3
E. lathyris(seeds)				40.0	40.0	<0.1	<2.0
E. lathyris(stem)				4.5	1.9	<0.5	2.0
E. marlothii	0.2	0.4	0.6	9.5	5.1	<0.5	3.3
E. tirucalli (UCB)*	0.07	0.13	0.2	5.0	2.4	<0.5	2.0
E. tirucalli (UCLA)†	0.1	0.3	0.4	8.5	4.4	<0.5	3.4
Hevea brasiliensis	1.3	0.2	1.5	9.6	5.1	<0.5	2.6
Jatropha curcas	<0.1	0.6	0.7	4.2	1.5	0.8	1.4
Monadenium rhizophorum	1.2	0.2	1.4	16.5	9.0	<0.5	6.0
Pedilanthys sp.	<0.1	<0.1	0.5	8.7	4.7	<0.5	2.3
Sarcostemma viminale	<0.1	0.8	0.8	12.3	6.6	<0.5	4.8
Synadenium grantii	0.4	0.4	0.8	15.0	6.6	<1.5	5.7

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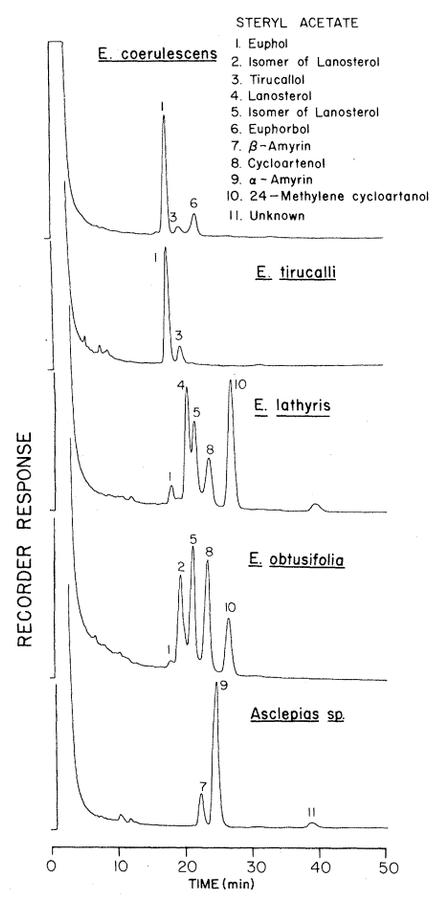
Table 2. Hydrocarbons and sterols from latex. Data shown are in percent of latex dry weight. The sterols were isolated from the latex by extraction with ten volumes of acetone under reflux. After filtration, the acetone was evaporated and the residue was acetylated with a mixture of acetic anhydride and pyridine (4:1) at 60°C for 1 hour. The sterols were identified by gas-liquid chromatography and gas chromatography–mass spectrophotometry (GC-MS) (Fig. 2). The residue from the acetone extraction was then extracted with hot benzene to give the rubber fraction, which was analyzed by NMR. Abbreviations are as follows. a, α-amyrin acetate; b, β-amyrin acetate; c, cycloartenol; d, euphol; e, euphorbol; f, fucosterol; g, isomer of lanosterol; h, isomer of lanosterol; i, lanosterol; j, 24-methylene cycloartanol; k, β-sitosterol; l, stigmasterol; and m, tirucallol

Source	Rubber	Sterols	Sterols identified (in order of abundance)*
Achras sapota	14†	66	a, b
Asclepias sp. (Brazil)	3.5	31	a, b
Asclepias sp. (United States)	(12)	(72)	a, b
Euphorbia characias			g, c, j, i
E. coerulescens	1	75	d, e, m
E. lathyris	3	50	j, i, g, c, d
E. misera			d, c, i, m
E. obtusifolia			g, c, h, j, d
E. tirucalli	1	50	d, m, e
E. trigona	1.5	75	
Hevea brasiliensis	87	1	k, f, l

*See also Ponsinet and Ourisson (6). †30 percent *cis*- and 70 percent *trans*-polyisoprene (by NMR). Other samples were all *cis*-polyisoprene.



well as from the work of Buchanan (*4*) and of Hall and Long (*5*), the reduced organic materials (those extractable by acetone and benzene) average 10 ± 5 percent. From the value of 10 percent "oil" content, the following rough calculation can be made: For biomass production an annual yield per acre of 10 tons (dry) can reasonably be expected (*2*), giving an oil production of 1 ton (7 barrels). The annual cultivation costs per acre are estimated to be $150 (*2*), or about $20 per barrel. Processing costs are not included, but neither is the value of the residual biomass. The possible added value of the oil for uses other than fuel has also yet to be determined. This preliminary cost estimate is close enough to the current price of petroleum to warrant further investigation. Test plantings in California of *Euphorbia lathyris* (of which we had sufficient seeds) and of *Euphorbia tirucalli* (whose cuttings were readily available to us) have been started, and from these we can try to confirm and refine the above figures. An experimental planting of *E. lathyris* at the South Coast Field Station of the University of California in Santa Ana has so far indicated a yield of not less than 8 barrels per acre in the 7-month growing season from February to September.

Table 2 shows that the latex of these plants is very rich in reduced photosynthetic materials (polyisoprenes and sterols), which comprise up to 80 percent of the dry weight (the latex, depending on the species and on the time of year and day, contains 30 to 90 percent water). Furthermore, some of the species are trees (such as *Achras sapote, Euphorbia tirucalli,* and *Euphorbia trigona*), and they could therefore be tapped. As compared to *H. brasiliensis*, which has to be hand-tapped, an automatic, continuous flow device (reducing the costs considerably) might be constructed for these species. Their latex contains much less rubber and more lower molecular weight material than *H. brasiliensis*, and therefore coagulates much less readily.

Although the results of this research are still fragmentary, the possibility of future "petrochemical plantations" is becoming apparent.

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Saccharin and Other Sweeteners: Mutagenic Properties

Abstract. *Saccharin preparations commonly distributed as artificial sweeteners exhibited mutagenic activity in bacterial tests. When administered orally to mice, mutagenic activity was demonstrable in the urines of these animals as well as in a host-mediated assay. Highly purified saccharin was not mutagenic in the direct assay, but the urines of mice to which this material had been administered exhibited mutagenic effects on one tester strain (*Salmonella typhimurium *TA100). Two other sweeteners, neohesperidin dihydrochalcone and xylitol, had no detectable mutagenic activity in any of these assays using his*$^-$ Salmonella typhimurium *strains TA100 or TA98.*

Although reports of 17 studies are available, conclusive evidence is lacking as to whether saccharin is mutagenic (*1*). This problem was reinvestigated by more sensitive methodologies (*2*) and tester strains—his$^-$ *Salmonella typhimurium* TA100 and TA98—developed by Ames *et al.* (*3*) for the detection of base-pair substitutions and frameshift mutations, respectively. Several saccharin preparations, as well as two other sweeteners, neohesperidin dihydrochalcone (NHDC) (*4*) and xylitol, were examined.

The following six saccharin preparations were tested: (i) a preparation, obtained from a local pharmacy, which is representative of the material distributed to the consumer as a pharmaceutical preparation (A); (ii) a sample manufactured by Sherwin-Williams Company and supplied by a wholesale pharmaceutical distributor (B); (iii) a sample, provided by Dr. D. Stoltz, from a lot (S-1022) that was used in a recent carcinogenic study (*5*) (C); (iv) a highly purified sample of C, also donated by Stoltz (D); (v) a powder containing 40 mg of saccharin and 1 g of lactose–potassium tartrate filler enclosed in a widely used and freely available convenience package (Sweet 'N Low) (E); and (vi) a sample, provided by Stoltz, from another lot (S-1233) used in the above-mentioned carcinogenic study (*5*) (F). Xylitol was supplied by Sigma, and NHDC was a gift from Dr. R. Horowitz.

Mutagenic activities were determined directly, as well as in the host-mediated assay, and in the urines after oral administration of the test substances, as in a previous study concerned with antischistosomal drugs (*6*). The direct assays of the compounds were conducted at several concentrations, both in the absence and presence of a rat liver microsomal fraction (S_9) (*7*). In the host-mediated assay, the bacterial tester strains were incubated for 6 hours in the peritoneal cavity of mice to which the test substance had been administered by gastric intubation (host-mediated assay) (*2*). The second type of assay in vivo consisted in the analysis of urines that had been collected for a 24-hour period after the oral administration to mice of the test compound. The mutagenic activities of 0.1- and 0.25-ml samples were assayed for each urine without prior concentration. All determinations were conducted in duplicate, and all observed positive mutagenic effects were dose-related.

After the oral administration of saccharin preparations A, B, and C, significant mutagenic activities in the urines were detectable with both *Salmonella* strains TA100 and TA98 (Table 1). Activities for TA98 were markedly enhanced after incubation of the urines with β-glucuronidase. In the case of TA100, unconjugated and conjugated mutagens were inactivated by liver microsome (S_9) preparations. By contrast, the mutagenic effects of the urines on TA98 were greater in the presence of S_9 (Table 1). Therefore, it would appear that two different mutagenic substances were present.

No mutagenic activity was found with TA98 in the urines of mice treated with the highly purified saccharin preparation D. However, there was low, but significant, mutagenic activity with the tester strain TA100. In addition, these urines contained a conjugated promutagen,