Agave Research Progress in Yucatan

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Abstract

The Center for Scientific Research of Yucatan carries out research aimed at solving some of the problems posed by the henequen industry in northern Yucatan. This paper briefly describes CICY's main research lines related to the hard fiber-producing agaves: a) taxonomic studies are being pursued to obtain a better understanding of the flora of the region; b) tissue culture techniques are used for the genetic improvement of agaves, and c) studies of composite materials and chemical substances derived from Henequen wastes are being carried out as possible alternatives to cordage production.

Introduction

From the second half of the 19th century the northern part of the Yucatan Peninsula became a major producer of hard fiber from the cultivation of Henequen (*Agave fourcroydes* Lem.). This industry reached its peak in terms of production and economic wealth during the early years of this century. The advent of synthetic fibers during the 1940's inverted this pattern, however, to one of poverty and social problems that have weighed heavily on the area ever since.

Because of its very poor soil conditions and its socio-economic problems (which are beyond the scope of this paper), it has not been easy to find a substitute for Henequen in the *zona henequenera*. The industry therefore persists, in spite of the fact that low market prices and demand give a low added value to the fiber and that Federal Government subsidies are needed to keep it going. The Henequen industry, which represents the only source of subsistance for some 60,000 families could, however, become far more prosperous than it is at present if production could be increased and alternative uses found for its fiber and wastes, the latter representing 96% of the total biomass of the leaf.

The Center for Scientific Research of Yucatan was created by the National Council for Science and Technology (CONACYT) in 1979 with the mandate to carry out basic reasearch on a number of topics centered around the local flora and to propose alternative uses for some of the main plants. Because of their importance in Yucatan, the hard fiber-producing agaves were the first group of plants to be singled out for close study and continue to play a major part in our research.

CICY is divided into four groups, namely Ecology, Applied Chemistry, Biotechnology and Genetics and Physiology which are doing research in the following areas: 1) biological studies, 2) alternative uses for fiber and agricultural wastes, and 3) genetic improvement.

In this paper we shall briefly discribe some of the work we have done at CICY concerning the Henequen problem and analyze the difficulties and perspectives which have emerged from it.

Discussion

Biological Studies. The Department of Ecology began studying the agaves of Yucatan in 1981, looking initially at their taxonomy. This group of plants has always posed problems for taxonomists because, according to Gómez-Pompa (1963), the genotype expresses itself in a wide range of phenotypic variants even within a single population, making it extremely difficult to distinguish between different species. Gómez-Pompa cites the following factors as causes of this variation: 1) high chromosome numbers, 2) polyploidy, 3) easy hybridization, and 4) efficient vegetative propagation.

The agaves of the Peninsula of Yucatan are no exception to this situation. The literature review brought to light wide discrepancies in the number of species reported for the region by different authors: the Maya nomenclature distinguishes 10 species; Roys (1931) reports five; Souza-Novelo (1945), eight, and Standley (1945) six. Amongst them there are two whose existence cannot be doubted: *Agave fourcroydes* Lem. and *A. sisalana* Perrine. However, there

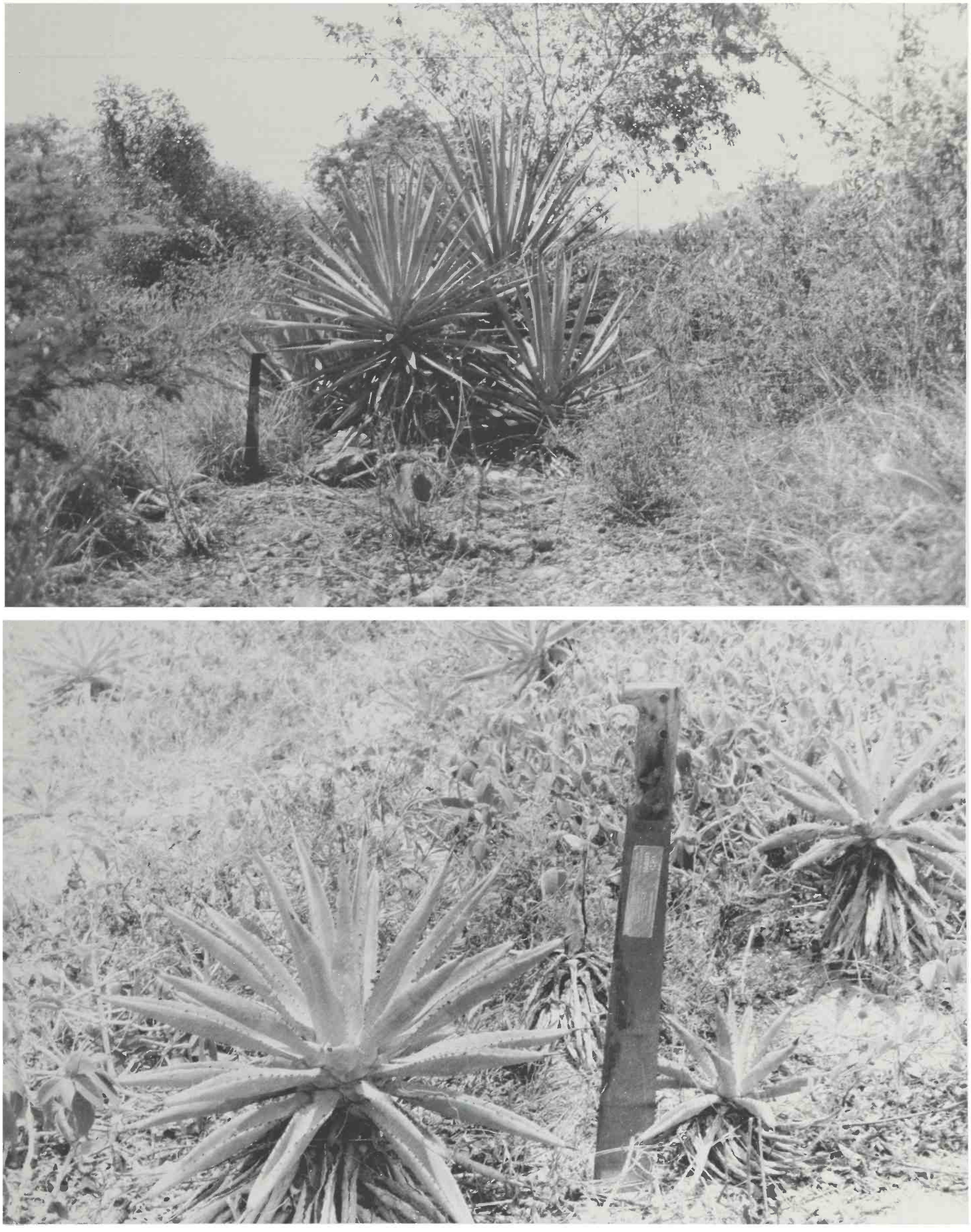

Figure 1. *Differences in the morphological expression of the complex* Agave angustifolia *in the peninsula of Yucatan are apparently related to the climate and type of vegetation:* **Above:** *A population in the semiarid zone of the northern coast near Rio Lagartos.* **Below:** *Part of a population of low deciduous secondary tropical forest in the central part of the peninsula near Carrillo Puerto. A wide range of intermediate forms has been found which makes it impossible to fit them into useful taxonomic groups.*

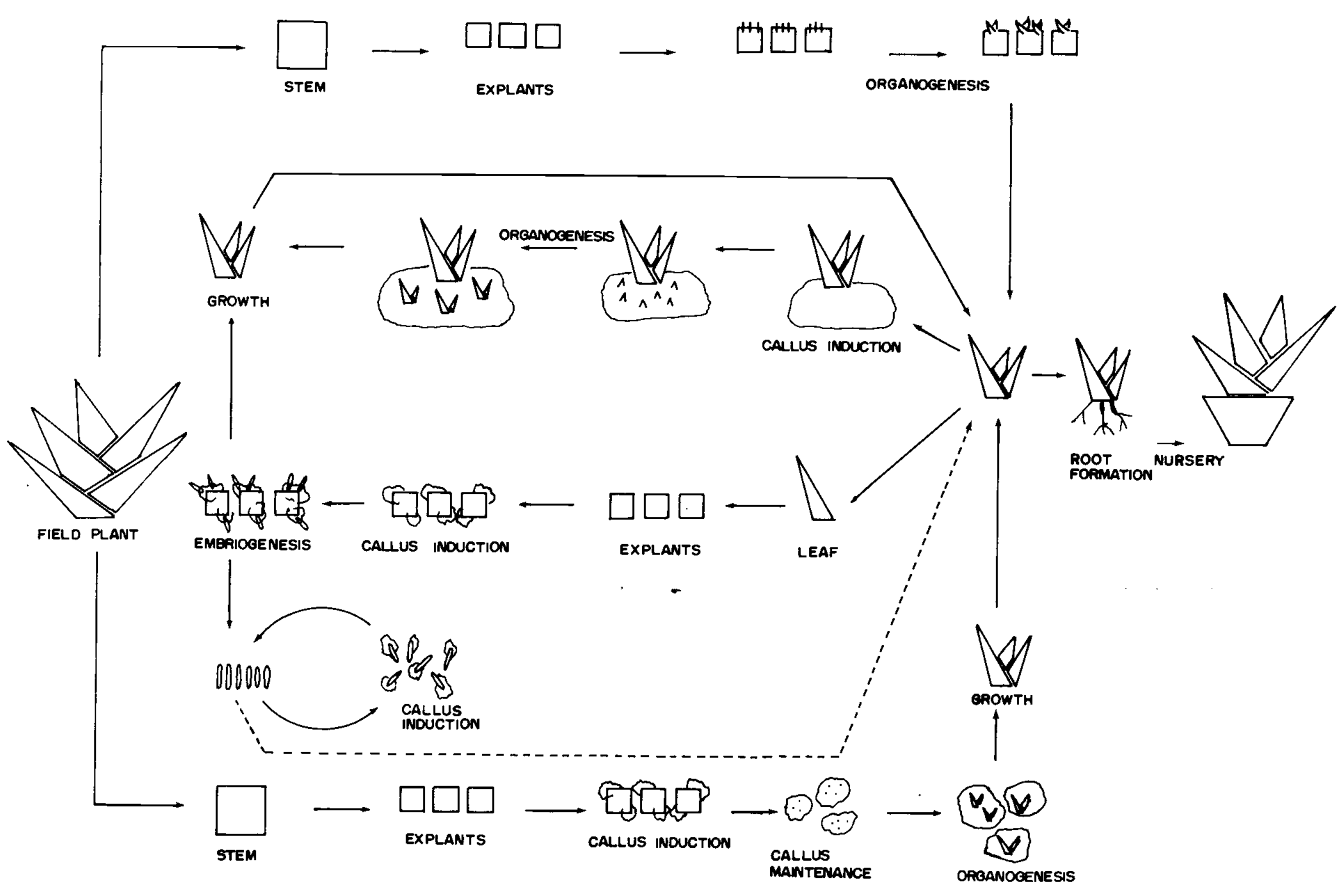

Figure 2. *Tissue culture of Henequen* (Agave fourcroydes).

are a great number of wild forms which do not fit the descriptions of either of these two species. Gentry (1982) has classified all of them as *A. angustifolia* Haw.

Originally, all the taxonomic studies were based on the morphological characteristics of the vegetative parts of the plants because, although they show the greatest degree of variation, they provide the basis for useful ecological descriptions. Orellana *et al.* (1981) found a wide range of phenotypic variation which seems to be largely caused by differences in the environment. For example, there is a direct relationship between the size of the plant and the climate and surrounding type of vegetation. The smaller forms are found in the semiarid zone along the northern coast of Yucatan, while the larger ones occur in the humid central and southern areas. In addition, microenvironments such as small outcrops of calcareous rock or areas which can be flooded modify the general pattern of variation.

The study of pollen grains has shown the same variation noted in the vegetative parts. Ludlow-Wiechers and Ojeda (1983) found that even when the ornamentation is constant there are five types of apertures, with as many as four different types being present in the same sample. The authors suggest that this variation is intimately linked to vegetative propagation and decrease in importance of sexual reproduction.

In his book *Agaves of Continental North America,* Gentry (1982) classifies all the wild forms of Yucatan as varieties of *Agave angustifolia.* Following Gentry's classification Orellana (1984) found two varieties of *A. angustifolia* in Yucatan *A. angustifolia* var. *marginata* Hort. and *A. angustifolia* var. *sargentii* Trel. He also mentions the existence of a third group which requires further study in order to decide whether it should be classified as an existing variety or should form a new one.

Orellana (1984) has also made a complete inventory of the species of the family Agavaceae found in the Peninsula of Yucatan. Following Hutchinson (1934), he reports the existence of 21 species belonging to nine genera and five tribes of which nine are native and 12 are introduced.

Ojeda *et al.* (1984) carried out the pollen analysis of the same species but their results correspond more closely with the classification proposed by Takhtajan (1980). They, however, recommend further studies of different genera of the same family and comparisons with genera from the Amaryllidaceae and Liliaceae before any definite conclusions are made.

Genetic Improvement Through Tissue Culture. Plant tissue culture techniques offer an alternative for those species which, because of their biological characteristics, are

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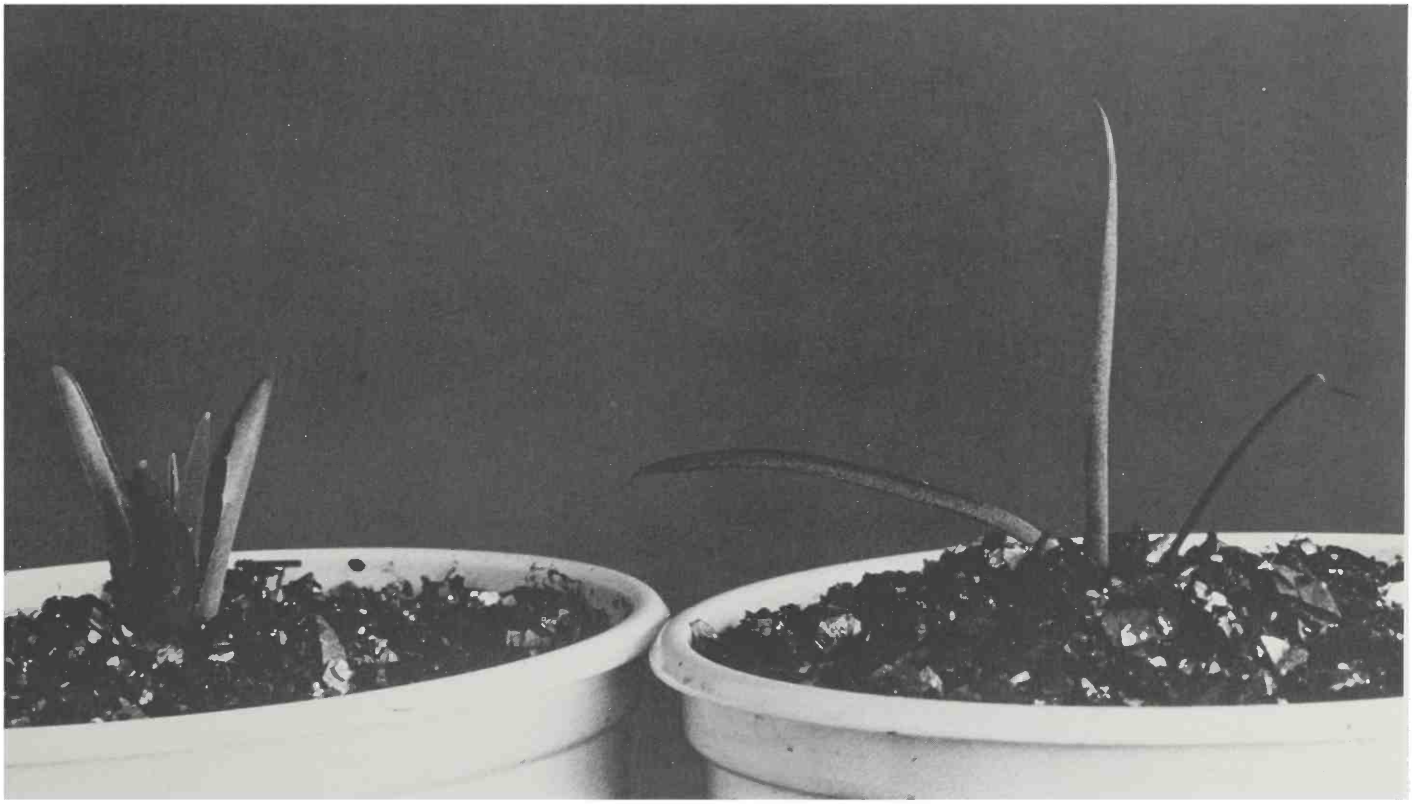

Figure 3. *Broad leaf morphological variant isolated from cultured cells of* Agave fourcroydes *compared with a typical regenerate from the same species.*

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difficult to study from a genetic point of view. Henequen, which only reaches sexual maturity after a period of 14 years and whose seeds have very low viability, is one such plant. Moreover, Henequen is far from being the ideal fiber producer; its 4% fiber content, low yield and late harvesting compare poorly with the characteristics of *A. sisalana* and the hybrid 11648 *(A. angustifolia* × *A. amaniensis* Trel. & Nowell back-crossed with *A. amaniensis)* grown in Tanzania (Lock, 1969). However, in spite of its poor showing, no one has ever attempted to genetically improve *A. fourcroydes,* probably because of the difficulties mentioned above.

Tissue culture studies started at CICY four years ago with the aim of improving the genetic variability of the hard fiber-producing agaves mainly through the fusion of somatic protoplasts.

We worked on all possible methods to culture and clone somatic cells and tried a wide range of nutritional, chemical and physical conditions without achieving much efficiency, though plants could be redifferentiated from callus induced from different explants such as meristems, leaves, rhizomes, etc. Finally, we discovered that the key factor for the successful culture of Henequen *in vitro* was the nature of the mother plant. If seedlings are induced directly from meristematic tissues and are grown on defined media under controlled environmental conditions (low light intensity), the cuttings obtained from the young leaves produce a highly morphogenic and embryogenic callus which rapidly gives rise to several dozen new plantlets. These are easily rooted and are transferred to pots with vermiculite before being transplanted to the nurseries.

Since protoplast work has been hindered by the very low efficiency of cell wall regeneration and division in our cultures, we have used the above material to evaluate the spontaneous variabliliy that arises in cultured cells.

In 1981 Larkin and Scowcroft found an enormous amount of variability in plants regenerated from cultured cells. This phenomenon, called somaclonal variation, has since then been confirmed in a wide range of species of both monocotyledonous and dicotyledonous plants.

The morphological variability of the plants, now readily produced by the micropropagation of tender leaf tissues, is being evaluated for the number of leaves, leaf size, rapidity of growth, number of marginal thorns, chromosome numbers, and odd phenotypes. These characteristics will be assessed for a period of two years since it appears that the enormous differences that emerge during the first three months in culture tend to homogenize at later stages. With regard to odd phenotypes only one such example with very broad and more numerous leaves has been isolated.

In order to increase the variability we have observed so far, we shall attempt this year to culture reproductive structures (anthers, pollen and ovaries) and will try to clone lines with

Table 1. Present CICY projects relating to *Agave fourcroydes.*

Composite Materials From Henequen Fiber
Physico-Mechanical Properties of Agave Fibers
unsaturated polyester
Felt-Polyester
felt-polyester-sand laminates
Gypsum (Fibrous Plaster)
short fiber-gypsum
pulp-gypsum
Hot Tops
Henequen fiber-coir fines/sand/synthetic resins
Cellulosic Derivatives
Chemical Pulp
cellulose triacetate
cellulose ethers
cellulose nitrate
Steroid Synthesis
Glucocorticoids From Industrial-Grade Hecogenin
Anti-fouling Agents
Descalants From Liquid Residues

Table 2. Comparative mechanical properties of different composites.

Property (kg/cm^2)	Henequen-Polyester-Sand	Fiberglass	Concrete
Tensile strength	150	812	17.1
Compressive strength	721	1,183	171.
Flexural strength	370	1,400	15.
Tensile modulus	10,175	34,000	204,082
Compressive modulus	114,230	65,000	—
Flexural modulus	130,346	59,500	—

gametic chromosome numbers for mutagenesis and polyploidization studies.

We are also engaged in the micropropagation of other agaves such as a variety of *A. letonae* Taylor ex Trel. from Guatemala which, under the conditions of cultivation in Yucatan, produces longer and more numerous leaves than *A. fourcroydes*, and the hybrid 11648 whose cultivation in Yucatan is prevented due to its sensitivity to *Phytophthora infestans.*

Alternative Uses for the Fiber and Waste Products. The Department of Applied Chemistry is actively working in two areas, polymeric materials and organic chemistry. An important part of the final objectives in each area is to develop technological alternatives that will make full and profitable use of the Henequen plant, through an intimate knowledge of its components.

Figure 5 describes the approximate composition of a Henequen leaf and some of the present and potential applications of the fiber and its byproducts. As in other agaves, a whole industry is dedicated to the transformation of the fiber into cordage goods, whose economics was severely affected by the appearance of the synthetic polymeric fibers on the market. In addition to this problem, it has been recognized (Lock, 1969) that only a small portion of the leaf is being exploited, while the rest is wasted. An exception to this in the Mexican Henequen industry is the utilization, albeit questionable, of part of the bagasse produced as forage for cattle, after mixing it with other ingredients. However, no other commercial processes are in operation at the present time and, moreover, not all of the fiber extracted goes into textile processing; at least 10% lacks the minimum length required to form twine. This residue is generally woven into low cost felt.

Several noteworthy efforts have been made to take advantage of the chemical structures present in the plant, which closely follow previous experiments carried out on sisal (Lock, 1969). Thus paper production from Henequen fiber has been examined in recent times as an alternative use that might meet the growing demand for paper in this country (Villalvazo, 1985), but the large industrial facilities required, and the potential pollution problems have caused the project to be temporarily shelved.

Based on the above premises, our Applied Chemistry Department has pursued four main projects, two on the fiber and two more on the liquid residues, or Henequen "juice." Table 1 lists an overview of these activities.

The fiber has excellent physico-mechanical characteristics (Cruz-Ramos, 1984), which makes it possible to obtain structural composite materials by embedding it into suitable matrices. Gypsum (plaster of Paris) and unsaturated polyester have been successfully reinforced by short fiber or felt, thus producing materials with improved mechanical properties. Polyester fiber composites, laminated on both sides with a sand coating, can withstand weather exposure and are apparently resistant to biological attack and are non-flammable making them potentially useful for a variety of building applications (Belmares *et al.*, 1981; Marchand *et al.*, 1985). Some of the mechanical properties of these materials are compared with those of fiberglass and concrete in Table 2. In addition to showing good mechanical characteristics, gypsum-fiber composites appear to be outstanding acoustic insulators, both absorbing and reflecting sound waves.

After several tests supervised by CICY, hot tops for steel ingot production, based on sand and natural fibers, to replace asbestos and glass fibers are already being used by a steelmaking company. Finally, polyurethane-Henequen composites are in the initial stages of a longer term development.

Regarding the use of Henequen fiber as a raw material for polymers, we have carried out chemical treatment tests on the short fiber and produced dissolving-grade pulps. This operation is possible because of the high alpha-cellulose content of the fiber (ca. 60% by weight) and its low percentage of lignin (ca. 13%). Although large amounts of hemicelluloses are present (ca. 16%), only minor processing difficulties occur as a result. The size of the manufacturing plant that would be required for this process is considerably smaller than that for papermaking.

The dissolving pulps can be transformed into a number of useful cellulose products, such as cellulose acetate and tri-

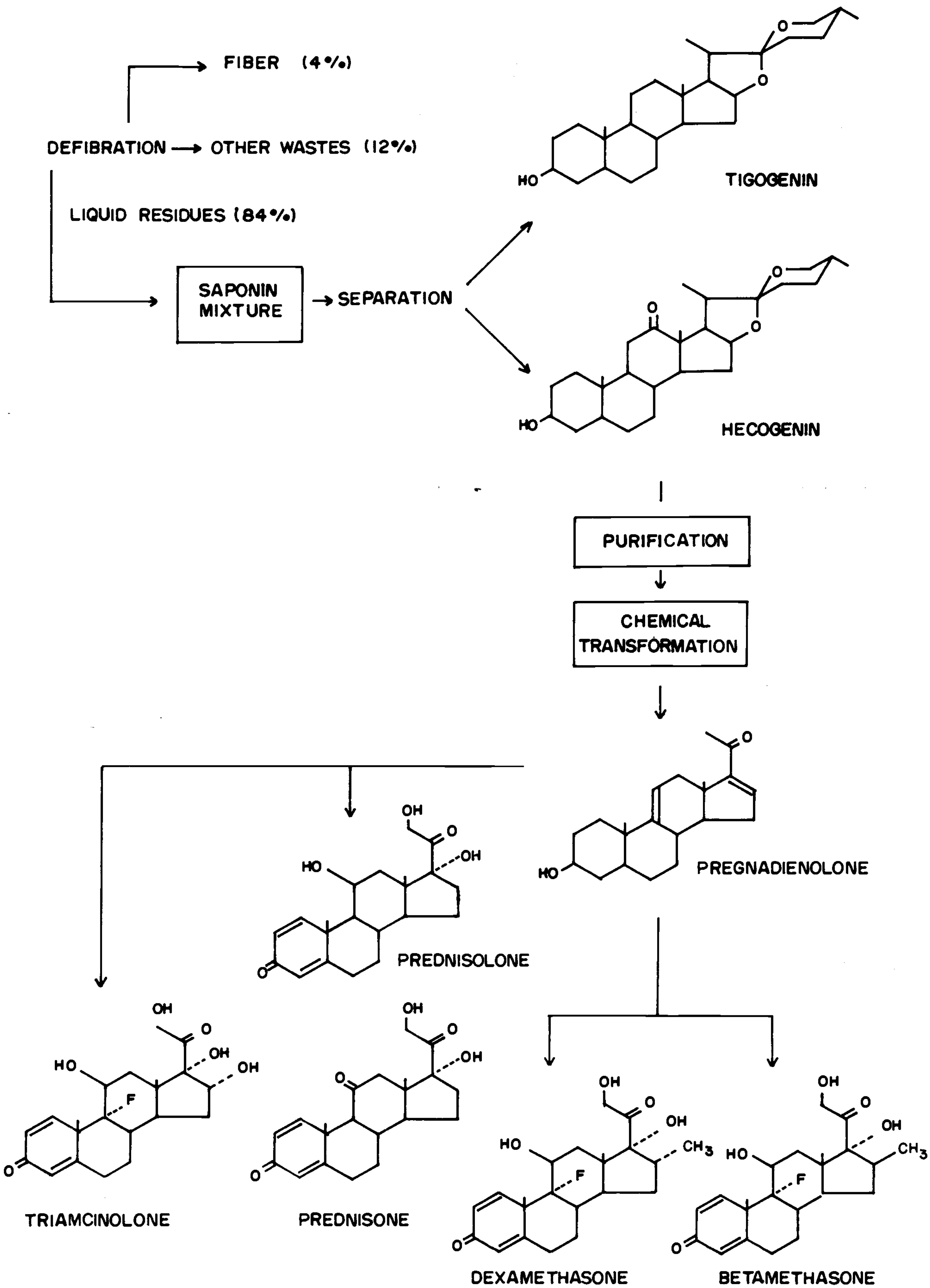

Figure 4. *Overall scheme of the advanced steroids produced using hecogenin as a raw material.*

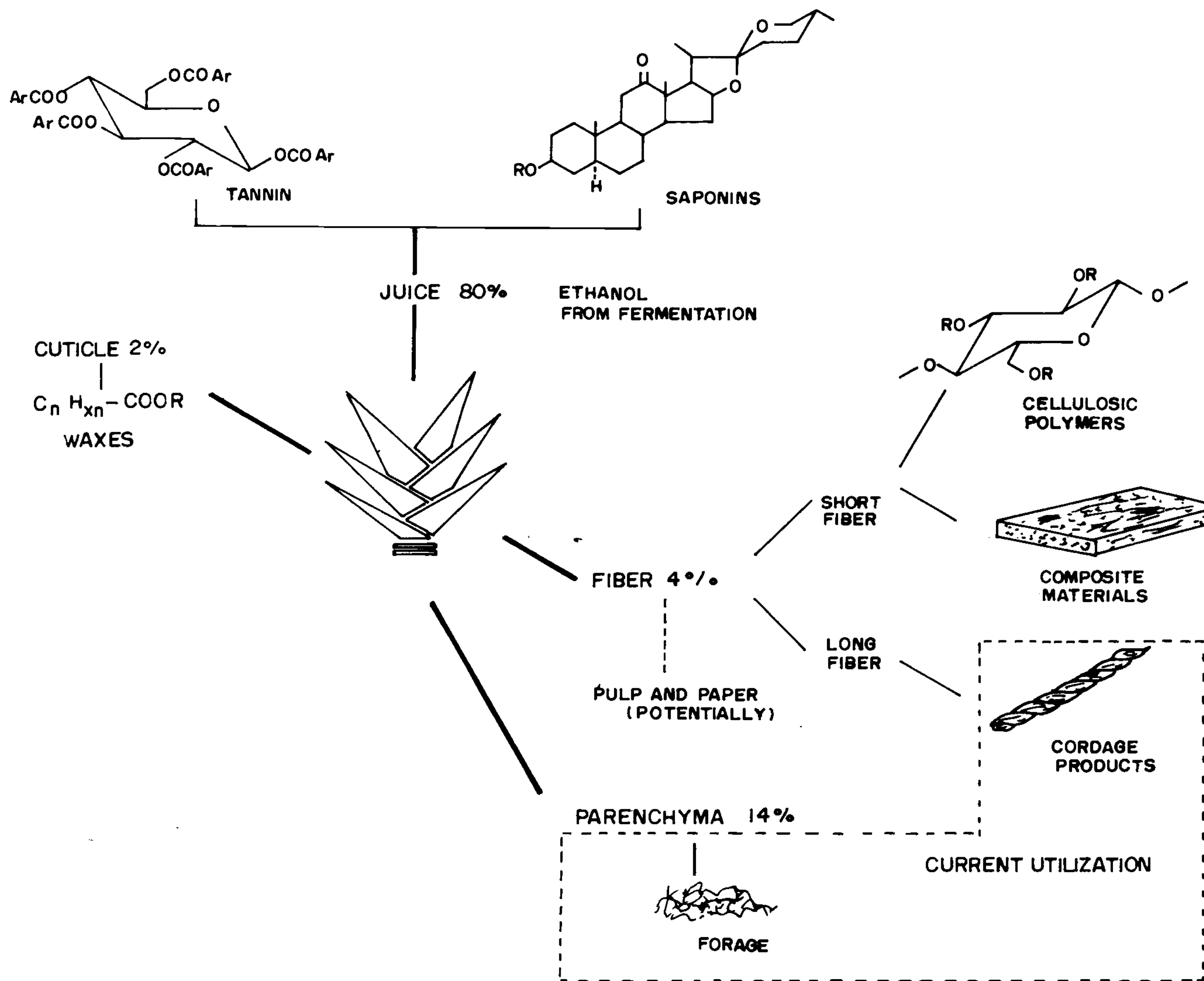

Figure 5. *Present and potential uses of the components of Henequen leaves.*

acetate, cellulose nitrate, carboxymethyl cellulose, and butyl cellulose, all of which are under study in our laboratory.

Advanced steroid synthesis from hecogenin (Azolkar *et al.*, 1979), has become a major target for the Henequen industry as an alternative with high added value for part of its liquid wastes. The process, as depicted in Figure 4 involves an initial step separating hecogenin from tigogenin, which are present on a 1:1 ratio in the leaf, along with small amounts of other saponins. Each of the outcoming fractions is enriched with one of the two main steroids, and chemical synthesis can then take place. CICY's Organic Chemistry Group is carrying out the study of different chemical paths to convert hecogenin into pregnadienone, which then becomes the key to the more advanced steroids, as shown in Figure 4.

The production of advanced steroids is attractive for several reasons, chiefly because of their potential use in highly active pharmaceuticals. For example, the anti-inflammatory activity of betamethasone and dexamethasone is 25 times that of hydrocortisone, whereas cortisone itself is only 0.8 times as active. Most significantly, few of the cortisone side effects have been detected when testing these products in humans.

Another possible use of the liquid residue or "juice" is a source of anti-fouling agents. At least one patent in the literature describes how to obtain anti-fouling formulations from sisal wastes after moderate chemical treatments and the addition of small amounts of metal complexing agents (Hughes, 1941; Rubin, 1963). The chemical moieties responsible for this apparent synergism have not been identified as yet; hence, a thorough chemical analysis and physico-chemical studies are under way to help understand the process and provide the basis for the formulation of anti-scalants.

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(D. Howell, pers. comm.). The apparently promiscuous Lechuguilla flowers do not seem unusual within the genus. The flowers represent an exceptional source of both water and energy for animals during the height of the dry season (May-June) when these resources are in short supply. Thus, animals are probably opportunistic, consuming a nectar which may not conform to their taste preference.

Amino acids are also found in Lechuguilla nectar in low concentrations. Both protein and non-protein amino acids were detected. While several amino acids were found in only one or two of the four plants studied, several were found in three or all four of them. These were phosphoserine, phosphoethanolamine, aspartic acid, serine, glumatic acid and glutamine, glycine, alanine, histidine, and threonine. These amino acids have also been found in the nectars of other agaves (Freeman et al., 1983).

Flower predation is an important aspect of the species' biology in the El Paso area. When we brought entire plants to the laboratory for nectar studies, we observed moth larvae climbing the shaft each evening, leaving a characteristic scar on the shaft, and feeding on unopened flowers. Field observations revealed 90-95% aborted flowers on shafts with scars. Specimens were submitted to the U.S. Department of Agriculture for identification. Their report indicates they belong to an unknown genus in the family Noctuidae, subfamily Amphipyrinae (D. M. Weisman, pers. comm.).

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