Diosgenin Production in North America

A Brief History

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Summary. Diosgenin is a steroidal aglycone occurring in certain species of *Dioscorea* native principally to eastern Mexico. In the 1940s, diosgenin became a much-sought-after intermediate for the chemical synthesis of certain corticosteroids and structurally related fertility regulants. Various difficulties of access to native sources led to attempts at plantation production. One of these, supported by the Upjohn Company between 1962 and 1980, was located on the Pacific coast of Guatemala and is described herein from the standpoint of technology development. The *Dioscorea* plant produces a long, coarse vine that requires support. The deep-growing, fleshy rhizome contains the diosgenin and, at harvest, must be removed from soil depths up to 1 m. Dry rhizome yield depends on supply of readily available (low-tension) soil water. Sites located over abundant water reserves give satisfactory rhizome yields, but diosgenin concentrations fall to uneconomically low levels under such circumstances. By 1980, diosgenin had been displaced by two products of soya oil processing, stigmasterol and sitosterol, which became available as a result of advances in microbial fermentation technology. Consequently, the cultivation of *Dioscorea* was abandoned.

The recent history of diosgenin production and use is marked by an unusual convergence of interests ranging from industrial botany through medicine, pharmaceutical chemistry, business, and international politics. It is a tale of extremes. Need for diosgenin arose precipitously in the 1940s, when no commercial source existed. It remained a favored

synthesis intermediate in the pharmaceutical industry for almost 40 years. During those years, great quantities were extracted from the rhizomes (root or tuber in trade parlance) of *Dioscorea* spp. native to and collected from the wild in eastern Mexico. Various pressures led to attempted cultivation of some of these species on plantations. Perhaps the most comprehensive and long-lived effort was that of the Upjohn Company in Guatemala. Under intensive cultivation, allowances had to be made for support of the long (up to 30 m) woody vines and for removal of rhizomes

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from soil depths up to 1 m at harvest. The most exacting site requirement was for a continuous and abundant source of low-tension soil water. Eventually, diosgenin lost its preferred position, so far as the Upjohn Company was concerned when stigmasterol and sitosterol became accessible microbially for the preparation of a variety of commercially useful steroid intermediates. As so often happens, displacement of a reigning botanical resulted from advances in industrial microbiology and in organic chemistry. But, while demand remained high, it would be difficult to name a more-challenging project in the field of industrial botany than was the attempted cultivation in the American tropics of the Mexican yam, *Dioscorea composita* Hemsl. (Matuda, 1953) and its variants.

Diosgenin is found in the storage organs (rhizomes) of certain species of *Dioscorea* that occur in Mexico and adjacent Guatemala. Chemically, diosgenin is the steroidal aglycone (Fig. 1), released upon acid hydrolysis, of a saponaceous glycoside, dioscin. Dioscin-containing plant parts, when macerated in water, impart a surfactant property that the indigens of Mexico and Central America employed for cleaning purposes. The starchy rhizomes were eaten, albeit without relish, in seasons of maize crop failure (de Landa, 1566). Dioscin is also hemolytic (Coursey, 1967). Pre-Columbian inhabitants of the region dispersed pulped rhizomes in slowly moving streams, whereupon fish suffocated, floated to the surface, and could be retrieved easily by hand. The archeological museum at the Tikal ruins in Guatemala contains an incised bone depiction of a Mayan fishing party removing very limp fish from foam-covered waters. More recently, North American patent medicines of the late 19th and early 20th centuries made use of dioscin-containing extracts in the concoction of remedies for "female troubles," but uses still to come could not have been foreseen by either stone-age Maya or our forebearers.

Diosgenin was isolated first from the rhizomes of an Asiatic species, *Dioscorea tokoro* Makino (Tsukamoto et al., 1936). Four years later, R.E. Marker (1940a, 1940b, 1943), of the Pennsylvania State College, published the molecular structure and botanical occurrences of this aglycone. Marker's interest went far beyond the academic to the realm of prescience. At that time, knowledge of the chemistry and physiology of mammalian hormones was expanding rapidly. Research quantities were needed of those constituents of the animal body that, up to that time, had been known only in trace amounts. Should therapeutic uses emerge, Marker reasoned, the pharmaceutical industry would be wholly unprepared to produce the bulk quantities

required. The small amounts then available were said to be under monopolistic control by three European manufacturers (see Senate Hearings, 1956). Only 3 years ahead of the crisis of supply, Marker et al. (1947) devised a synthesis of progesterone, the *corpus luteum* hormone, from diosgenin. On a hunch (Marker, 1987), he explored the *Dioscoreaceae* of eastern Mexico, where he found substantial quantities of diosgenin-containing raw material. His successes in both chemical and plant exploratory facets of this field have won for him far greater attention in Mexico (Lehmann et al., 1973) than they have in his native land. He extracted diosgenin from the woody rhizomes of the "cabeza de negro," *Dioscorea mexicana* Guillemain, and prepared from it 3 kg of progesterone (Marker, 1987), by far the largest quantity ever before

seen. Marker had not long to wait. In 1949, two developments in medicine set off the explosive demand that he had foreseen. From the Mayo Clinic there came an announcement of the dramatic effect of administered cortisone upon symptoms of rheumatoid arthritis. And, from the Worcester Inst. for Developmental Biology, came the first practical fertility regulants. Although the earliest intermediates for cortisone synthesis were the steroidal bile acids, both cortisone and the fertility regulants could be made, at that time, much more easily from diosgenin. Consequently, a world-wide search began for abundant and inexpensive supplies of intermediates, including diosgenin, that could be used advantageously for the manufacture of desired steroid compounds (for two interesting examples, see Landrum, 1986).

Total synthesis of steroids from the simplest organic compounds was still years in the future—and that route would be prohibitively expensive in any case. In the words of George Pucher, at one time of the Connecticut Agricultural Experiment Station, New Haven, the plant world came to be regarded as a storehouse of rare chemicals, and it was ransacked from top to bottom in search of ever more useful intermediates. Marker scored a third time by discovering, also in eastern Mexico, enormous populations of *Dioscorea composita* Hemsl., known locally as "barbasco," a far more tractable source of diosgenin than was *D. mexicana*. Mexican resources began to be "mined" by rhizome collectors. Mexican authorities estimated that the states of Vera Cruz and Oaxaca alone contained up to 25 t of dry root equivalent/

Fig. 1. *Diosgenin*.

km², and that $\approx$ 5500 t of air-dried material was then being removed annually (Senate Hearings, 1956). Giral (1958) stated that the diosgenin concentration in this material ranged between 3% and 4% on a dry basis, despite many more optimistic reports. As a consequence of Marker's work, and of continuing improvements in chemical technology, the price of progesterone to manufacturers of hormonal products fell from about \$200/g in 1940, to \$80 in 1943, and to 30¢ in 1955 (Senate Hearings, 1956). Owing to its fortunate position with respect to the geographical distribution of diosgenin-containing species, Mexico became the world capital of the steroid intermediates industry, a position that it held for more than 20 years. A parallel, although much smaller, industry sprang up in neighboring Guatemala that was based on the occurrence there of *D. flori-*

Fig. 2. Vine canopy on post and wire support, central farm, Guatemala.

bunda Mart et Gal., a less vigorous species possessing a higher average diosgenin content ($\approx$ 5%) and recognized by the yellow flesh of its rhizomes (those of *D. composita* are white). Substantial quantities were processed by the Compania Agricola Industrial Guatemalteca (CAIGSA).

Ultimately, Mexico lost its preferred position as a consequence of several ongoing factors. First, continued exploitation of natural stands of *Dioscorea* led to ever higher transportation costs as distances between jungle and processing centers increased and as second-growth stands suffered in terms of yield per unit area of terrain and of assay. Second, other intermediates were brought along, including stigmasterol and sitosterol from soya oils and hecogenin from sisal wastes. The third factor was Mexico's attempt to protect its national industry in a classic parallel to the earlier attempts of Peru to preserve *Cinchona* bark production in the Andes and of Brazil to prevent loss of *Hevea* rubber to foreign planters. Political repercussions of Mexico's efforts in this direction were aired before a Senate Subcommittee on the 'Wonder Drugs' (1956) chaired by Sen. Joseph O'Mahoney. Testimony before this group affords a broad insight concerning the technologies, the botany, and chemistry, as well as the business and international trade aspects of a new and turbulent industry.

Inevitably, commercial-scale cultivation of suitable *Dioscorea* spp. was contemplated by those firms unable to gain unrestricted access to Mexican raw materials and by Mexican firms concerned about the uncertainty of future supplies. Edible yams of the same genus had been cultivated for a very long time in Africa and the Indies, and considerable technology had accumulated (Coursey, 1967). The earliest attempts to cultivate genin-containing yams in the western hemisphere are believed to have taken place in Guatemala (Bruhn et al., 1972), in Costa Rica and in Puerto Rico. One of the more systematic and comprehensive efforts was that of the U. S. Dept. of Agriculture in Mayaguez and another at the Puerto Rican Experiment Station in Rio Piedras. The first Guatemalan and Costa Rican attempts were terminated prematurely. Yield extrapolations from field plots to commercial-scale plantings were published by Puerto Rican workers (Martin et al., 1966), with recommendations for cultivation. It is understood that Irving Sollins, one of the more knowledgeable witnesses before the O'Mahoney subcommittee, formed a company to make use of Puerto Rican technologies. However, the clay soils and inclement weather combined to make harvesting both difficult and costly. An earlier journal paper

(Kennard and Morris, 1956) had already encouraged those who would attempt cultivation under more favorable conditions for harvesting activities. In 1957, Percy L. Julian, well-known organic chemist and my one-time mentor in that subject at DePauw Univ., came to New York to discuss possibilities for growing *Dioscorea* successfully in Guatemala, where I had some experience with the introduction of Cinchona. On the evidence available, a rough estimate of 60% in favor of success was enough to set Julian on a course of action. For the purpose, he formed Empresa Agro-Quimica Guatemalteca, S.A., whereupon I began a 20-year-long schedule of commuting between New York and Guatemala.

Empresa's first plantings were made late in 1958, employing material purchased from B.A. Krukoff of Ensayos Agricolas, S.A. (Landrum, 1986). First harvests were disappointing. The project survived only because one field or block on the farm yielded at or even above our best expectation. We took this exception as a measure of species potential and so defined our task in terms of learning how to achieve the latter routinely. In 1962, the Fine Chemicals Division of the Upjohn Company acquired the project, moved it to an area of low-bulk-density soils that were derived from recent volcanic ash falls (see Dawson et al., 1980), and proceeded patiently to develop technology. This is designated herein as the central farm. Production objectives were set minimally at 18 t·ha⁻¹ of dry rhizome material assaying 4% diosgenin, all within a 3-year crop cycle. There was reason to believe that these figures might be improved to as much as 28 t of dry material with a content of 5.5% to 6.0% diosgenin. Harvested crops on the new site gave on average 4% of diosgenin, but dry rhizome yields fell to 50% of the minimum expected or less. Consequently, a three-tiered experimental approach was devised to identify the limiting factor(s) and means for rectification. Field plot tests employing the usual management variables failed to affect either growth rates or diosgenin concentrations. Off-farm tests scattered from Florida to Costa Rica gave clear indication that this heretofore wild plant required unusually great quantities of soil water and that this water must be within reach of the long coarse roots but not the rhizome meristems. A mini-scale greenhouse experimental system that measured relative growth rates of very small tubers formed by young seedlings not only confirmed interpretation of the site testing outcome, but also gave an explanation. That was, *D. composita* rhizome growth rates depend linearly upon the supply of readily available (low-tension) soil water (Dawson et al., 1980, 1983).

Three site categories well-suited to rapid

Fig. 3. Parts of an unusually large tuber weighing 45 kg fresh, taken from a planting near Retalhuleu, Guatemala.

growth of *Dioscorea* rhizomes in terms of dry matter accumulation were identified by the above means. The first occupied benches of alluvium above the flood plains of rivers such as the Motagua in Guatemala and the Aguan in Honduras. The second lay over relatively shallow permanent water tables such as the black sands below Tiquesate, Guatemala, or the peats of the Florida Everglades. In both places, water tables could be controlled by dry-season irrigation or by poldering and pumping. The third was characterized by a friable topsoil over a barrier to percolation. Old marine sediments (Bucul clay with 14% organic matter in the topsoil) near Retalhuleu, Guatemala, and an old lava flow from volcano Turrialba in Costa Rica, were representative. The principal point is that subsoil profile with respect to the storage and facile delivery of water to yam roots is much more important to the growth of this crop than is topsoil classification. Gross yields on each of these sites came close to the initial objective. For example, 32 ha of cotton land below Tiquesate gave 15 t of dry rhizome/ha in 3 years. In all such cases, however, diosgenin concentration (assay) in the dry material fell to 3% or lower. Because of this totally unexpected development, estimated diosgenin yield equivalents at Tiquesate, 400 kg·ha⁻¹ in 3 years, fell slightly below those (450-kg equivalents) on the central farm. This negative relationship between vigorous rhizome growth and high

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rhizome yield on the one hand, and diosgenin concentration and yield on the other was approached by a program of single-plant selection for high assay, followed by clonal propagation. Only two such clones materialized. While assay remained elevated (5.5% to 6.0%) on drier sites, it fell to 3% on the wetter sites, where rhizome yields were high. When plants of a large population of non-selected material were sampled and assayed singly, and those assaying above the mean were cloned and reassayed, the two frequency distribution curves were identical. Thus, genetic potential for solving the overall problem did not appear to be great.

For 20 years, production of rhizomes on a commercial scale was maintained along with the research program. Some 3000 t assaying 4% on average were accumulated. In 1960 U.S. dollars, the diosgenin equivalent of dried rhizomes in Mexico cost \$6.20/kg. By planting between rows of young *Hevea*, Julian hoped to reduce the cost to \$1.20 to \$3.20/kg. In 1966 dollars, the data of Martin et al. (1966), when extrapolated to our circumstances, allowed an estimated production cost of \$2.40/kg equivalent. In 1973, sales of our dry rhizome materials to Mexican and Guatemalan processors brought an average of \$10/kg equivalent. Rhizomes produced on the central farm cost \$44/kg equivalent of diosgenin in 1977 dollars, while, at Tiquesate, the figure was \$42. These figures include costs of research. In 1979, mainland China began offering diosgenin in bulk at \$30/kg.

In retrospect, most of the early assumptions upon which cultivation attempts were based proved to be wholly inadequate. The difficulties arose from a combination of unusual botanical characteristics of the plant and the intensity of the lowland tropical environment. Not only were the physiology and biochemistry of the plant unknown, but the developmental morphology, and even the taxonomy, were poorly understood. For example, crop logs could be prepared only with greatest difficulty. Tuber weight variances within samplings were enormous, while means and modes of sampled weight were skewed heavily toward lower values. Assay data varied so widely within samples drawn from a single population that values reported in the literature and based on small numbers of plants collected in the field had to be regarded with extreme caution. At least a portion of the variation in diosgenin content was likely caused by fluctuations in starch content. In any event, by 1980, diosgenin had run its course so far as the Upjohn Company was concerned. Mountains of by-product sitosterol yielded to microbial modification and thus made obsolete any further attempt to seek an agronomic solution.

In summary, the key to reasonably ac-

ceptable yields of genin-containing *Dioscorea* rhizome was found in an obscure and long-neglected aspect of plant physiology, namely, the interactions between roots and soil environment at depths of 1 m or more. That accomplished, a negative relationship between rhizome growth and diosgenin concentration in the rhizome blocked further progress. It is difficult enough to influence root environment 1 to 2 m down, but the latter perversity of nature could not be overcome. Considering the enormous number of complex problems that they faced over a period of 20 years, it is a tribute to the people who spent their best years trying that the project came as near to economic viability as it did.

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SCHEDULE of FUTURE ANNUAL MEETINGS of the AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE

No.	Date	Location	Comment
89th	31 July-6 Aug. 1992	Sheraton Waikiki Hotel and Sheraton Princess Kaiulani Hotel, Honolulu, Hawaii	Theme is "Pan-Pacific Horticulture." To be hosted by horticulture faculty at the Univ. of Hawaii, Honolulu.
90th	24-29 July 1993	Opryland Hotel, Nashville, Tenn.	To be hosted by the ornamental horticulture and landscape design faculty and the plant and soil science faculty at the Univ. of Tennessee, Knoxville.
91st	7-11 Aug. 1994	Oregon State Univ., Corvallis	To be hosted by the horticulture faculty.
92nd	30 July-3 Aug. 1995	Montreal (Que.) Convention Centre and nearby hotels	The Canadian Society for Horticultural Science will meet jointly with ASHS.
93rd	6-10 Oct. 1996	Lexington (Ky.) Convention Center and four nearby hotels	To be hosted by the Dept. of Horticulture and Landscape Architecture at the Univ. of Kentucky.
94th	10-14 Aug. 1997	Cervantes Convention Center, St. Louis (Mo.) and nearby hotels	To be hosted by the Dept. of Horticulture at the Univ. of Missouri-Columbia.
95th	8-12 Aug. 1998	Raleigh (N.C.) Civic and Convention Center and nearby hotels	To be hosted by the Dept. of Horticultural Science at North Carolina State Univ.