Upjohn Stigmasterol and Sitosterol Extraction Team (Greiner Team)

Invented a leaching/countercurrent crystallization process to recover both stigmasterol and sitosterol from soy sterols [a distillative byproduct from purification of soybean oil consisting of stigmasterol (~20%), sitosterol (~40%), campesterol (~20%), brassicasterol (~5%) plus others]. Invention provided valuable starting materials used for steroid medicine synthesis to be recovered from a sustainable, readily available, inexpensive plant source.

Here's what happened:

1940's: Percy Julian observes that addition of water to soy sterols promotes crystallization of stigmasterol

early 1950's: Upjohn chemists (Herr, Heyr, Centolela) discover and refine a commercially viable route for chemical synthesis of progesterone from stigmasterol; Upjohn replaces progesterone obtained from Russell Marker's Syntex Co. (synthesized from diosgenin recovered from Mexican yams), with progesterone made internally from soy-sourced stigmasterol.

1955: Greiner Team invents an innovative counter-current crystallization process for recovering high-purity stigmasterol from crude soy sterol mixtures in ~20% mass yield. Upjohn switches to making all of its own progesterone from stigmasterol recovered from soy sterols via the Greiner process.

The remaining ~80% mass of sterols (predominately sitosterol) is also recovered from the Greiner process; Upjohn starts stockpiling the mixture on the Kalamazoo manufacturing site.

Late 1960's: formalizing Greiner's prompting to find a use for the >10 year stockpile of sitosterol rather than discard it, Upjohn forms the sitosterol utilization project to develop a use for the material.

1974: Wovcha Team discovers mutant strain of *Mycobacterium fortuitum* that converts sterols with fully saturated sidechains (like sitosterol and campesterol) to 9α-hydroxyandrostendione, which can be used as an intermediate for steroids manufacture, and the recovered sterols become a second valuable starting material obtained from soy sterols.

What has been said about the significance of Greiner's process and Upjohn's use of stigmasterol and sitosterol

"Upjohn's J. Ward Greiner, a chemical engineer, began working part-time on his own initiative to develop counter-current distribution techniques to separate stigmasterol from sitosterol. [Photos] of the discarded sitosterol stockpile graphically demonstrate that he succeeded, and dramatize the magnitude of stigmasterol utilization that followed.

The growing stockpile, looking like the Badlands of South Dakota, fueled another Greiner dream, the utilization of sitosterol, for which he became the chief advocate. By the late 1960's the discarded sitosterol stockpile had become enormous. The sitosterol utilization project, based on chemical or microbiological methods, or utilization per se, was formally initiated in the early 1970's. The outcome was just as dramatic as the events that had brought Upjohn to this point in the first place. The sitosterol pile was resurrected by the discovery and development of yet another combination of microbiological/chemical processes.

from: J.A. Hogg. Steroids, the steroid community, and Upjohn in perspective: a profile of innovation. *Steroids*, 1992, 57, 593-616

"Following Russell Marker's conversion of steroidal sapogenins to progesterone, Mexican diosgenin became the prime starting material for sex hormones, the contraceptive progestogens, the corticosteroids and the sterol diuretics. The microbiological oxygenation of the 11 position of progesterone by Upjohn in 1956 turned diosgenin into the leading corticosteroid raw material via its conversion of pregnenolone, progesterone and other intermediates such as Compound S. Simultaneously, however, Upjohn developed an economic process for separating sterols into sitosterol (80%) and stigmasterol (20%). The latter was used to prepare progesterone and the former stored for some future use. Now stigmasterol is the leading raw material for the progesterone-corticosteroid process and a use has been found for the by-product sitosterol."

from: N. Applezweig. Steroid Drugs From Botanical Sources: Future Prospects. Renewable Resources – A Systemic Approach. Edited by Enrique Campos-López. Academic Press, New York, 1980.

“The efficiency of this progesterone-based process was further enhanced by the work of W. Greiner and G. Fevig, who devised a countercurrent crystallization method for isolating the stigmasterol fraction from the soy sterols. The residue from this separation, composed of approximately 80% of the original soy sterol mixture, was stockpiled in the back of the Upjohn plant in the form of 3000-pound “pigs”. Of course, it was hoped that someday a method for the utilization of the sitosterol fraction might be developed to capitalize on this by-product of a by-product. As Upjohn grew to become the world’s largest producer of corticosteroids, this sterol accumulation eventually took on the dimensions of a small mountain, a particularly prominent landmark in the surrounding flat Michigan landscape.
The key to sitosterol utilization required the efficient degradation of the unfunctionalized C-17 side chain. Chemical means of accomplishing this are still not good enough; however, microbiologists were able to develop microorganisms that are capable of selectively degrading the side chain.”

From: D.A. Livingston. Application of Silicon Chemistry in the Corticosteroid Field. Advances in Medicinal Chemistry, volume 1, pages 137-174, 1992.

Team Published Paper

A. Poulus, J.W. Greiner, G.A. Fevig. **Separation of sterols by counter-current crystallization.** *Ind. Eng. Chem.*, 1961, 53, 949-962.

Team Patent

J.W. Greiner, G. Fevig. **Countercurrent extraction of sterols.**
U.S. Patent 2,839,544. Filed 4 Sep 1956, granted 17 Jun 1958.