Steroids, the steroid community, and Upjohn in perspective: a profile of innovation

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The announcement in 1949 at the Mayo Clinic of the dramatic effect of cortisone in alleviating the symptoms of rheumatoid arthritis triggered a competitive worldwide research and development effort directed toward a single goal, the practical synthesis of the rare corticosteroids. The confluence of an extraordinary coalescence of multiple events and circumstances in the growth of the Upjohn Company with the Mayo discovery, inclusive of a pioneering role in the steroid field, conspired to create an environment ripe for innovation. The breakthrough, which gave Upjohn an early competitive edge, followed with startling swiftness. A common mold of the genus Rhizopus was found to introduce enzymatically an 11α-hydroxyl group directly into the female hormone progesterone, which had just been synthesized from the soybean sterol stigmasterol—a one-step solution to the known multistep alternatives for 11-oxygenation. Retrospective analysis of this event in perspective with other key developments before and after at Upjohn and in the steroid community reveals a striking profile of ongoing innovation. A parallel scenario in kind was repeated at Upjohn a quarter century later. The sister soybean sterol sitosterol was radically degraded microbiologically and concurrently oxygenated in ring C to produce 9α-hydroxyandrostenedione, an alternative key intermediate for corticoid synthesis. New chemical processes, highly integrated with existing processes, assured the continuation of Upjohn's leading role in steroid hormone production. (*Steroids* **57**:593–616, 1992)

"The journalistic tradition so exalts novelty and flashy discovery that standard accounts for the public . . . miss the usual activity of science . . . and . . . convey a false impression about what drives research."

Stephen Jay Gould,
Wonderful Life: The Burgess Shale
and the *Nature of History*

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Introduction

In my view, two important discoveries (Chart 1) were responsible for the tremendous acceleration of research on steroids that occurred in the 1950s and beyond, the so-called golden age of steroids.

The first of these was enabled by a supply of costly ox bile–derived corticosteroids from the pioneer-spirited Research and Development Department at Merck. Hench et al. at the Mayo Clinic announced in April 1949 that cortisone is useful in treating the symptoms of rheumatoid arthritis,[1] a discovery of heroic proportions. The other discovery was the demonstration in animals by Pincus et al. at the Worcester Foundation of the potential of the female hormone progesterone as a contraceptive agent.[2] The latter discovery unfolded

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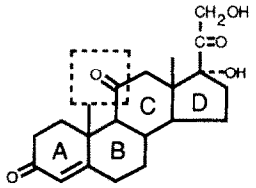
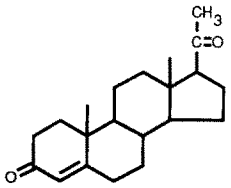


(1)

Cortisone

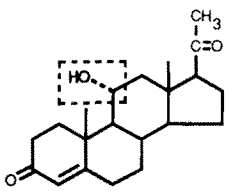
(Rheumatoid Arthritis
Hench et al., 1949) [1]



(2)

Progesterone

(Contraception,
Pincus, 1950)[2]



(3)

11α-Hydroxyprogesterone

(Peterson and Murray, 1952) [3,4,5]

Chart 1

less dramatically, did not identify a specific drug entity, and was muted by the potential for controversy.

The ensuing burst of activity throughout the already competitive steroid community was unique in steroid history; now the participants were driven by the certainty of return on investment in medically important areas and everyone had an even start—albeit from different perspectives—which made the sense of competition even more acute.

Several curious coincidences connect these two developments. Both discoveries were based on concepts of rational design, each of which was derived from observations in pregnant females; in both situations, to become of practical value, a substantial obstacle had to be surmounted. In the first instance, the 11-oxygen of ring C of the rare adrenal hormones, such as cortisone, is not found in any of the abundant steroids in nature from which it might be synthesized, thus limiting its synthesis to the use of steroids with functionality in or near ring C and adding to the complexity. In the second instance, progesterone is inactive orally, which would complicate its use as a contraceptive. As it turned out, the female hormone progesterone, in addition to pointing the way to the contraceptive pill, also became the key intermediate at Upjohn for the synthesis of cortisone and other corticosteroids.

The latter most curious of the coincidences connecting these two hormone classes came about by the history-making discovery at Upjohn, reported by Peterson and Murray[3] in 1952 that common molds of the *Rhizopus* genus are capable of introducing oxygen by fermentation procedures directly into progesterone at the otherwise elusive 11-position,[3-5] a dramatic breakthrough in itself. 11α-Hydroxyprogesterone[3,4] (Chart 1, 3) thus became available from progesterone in high yield. The stage was set for a redirected chemical effort with a new challenge to complete the synthesis of cortisone and hydrocortisone.

Following is an account of the history of steroids at Upjohn, reconstructed in the context of the title and in chronological order from published literature, extant documents, extensive testimonial, and recollections of the author as a colleague or acquaintance of the participants.

We will see that the simplistic, single-hero story, as it has been portrayed, of this microbiological discovery is a myth; and that faced with fierce competition in the steroid community, Upjohn's initial competitive advantage was not to be sustained on the merits of *Rhizopus* alone.

Precortisone history

How and why did Upjohn acquire such a commanding lead so soon after the Hench et al. announcement of cortisone's great value? Given that either chance or creative innovation by individuals is immensely favored within a "prepared institutional environment," it is important first to review the factors that shaped such an environment at Upjohn. That setting in 1949 had direct bearing on this important discovery, as well as the uncannily timed capability to exploit it and the continued innovation that was to follow in order to maintain the initial advantage.

It was Dr. F. W. Heyl (Figure 1), the first Research Director (1913–1944), who initiated the company's pioneering endocrine research program in 1933 via the strategy of hiring 10 postdoctorates from major universities, many of whom had conducted research in the endocrine field and afterward returned to academia. This set a pattern of professional acquisition; scientists trained and/or inspired by leaders in the hormone field were attracted for three decades in an unbroken skein.

By the late 1940s, Upjohn had products in all four steroid hormone classes (Chart 2), largely extracts from animal tissues, plasma or urine, and hormone derivatives. Of particular interest are the adrenal cortical extracts, the first to be adrenaline free, which were used to sustain life in instances of adrenal insufficiency. All four hormone classes continued to assume important roles in their respective medical arenas, still evolving even today—including the analogs of testosterone, the anabolic steroids whose misuse by athletes and labeling as "steroids" by the media has created an

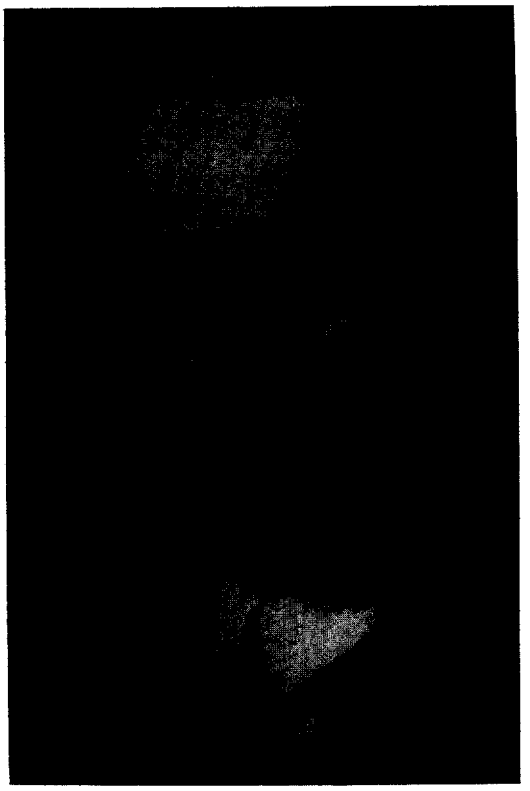


Figure 1 Frederick W. Heyl.

unfortunate negative public perception of all steroid hormones.

In connection with the adrenal gland work, Kuizenga, one of the postdoctorates, and Cartland (Figure 2) participated in the worldwide thrust in 1935–1940 to isolate and identify the 40 or more crystalline substances of the adrenal gland, one of which was cortisone. By 1949, Upjohn's commitment to the hormone field had become well entrenched. The isolation, structure determination, synthesis, and endocrine characterization of steroid hormones had been highly competitive activities worldwide since the early 1930s. Dwight Ingle, one of the later postdoctorates, gave endocrine assay support to the development of adrenal products, and had established a worldwide reputation in this field.

In 1945 a major plant expansion project, fourfold greater than the foreseeable need, was started at the initiative of President Donald S. Gilmore (Figure 3), a man of extraordinary vision and action, clearly setting the corporate sights on becoming a major pharmaceutical force. It was completed in 1950, just in time to cope with the expansion of the new steroid product line, which could not have been foreseen.

Brand new fermentation and chemical production facilities were constructed during this same 5-year period as can be seen at this site, shown then and now (Figure 4). The fermentation capability stemmed from the World War II effort in antibiotic research. The

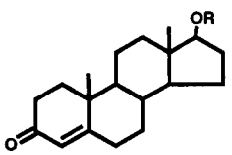
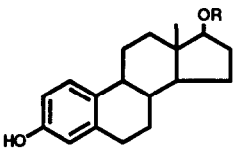
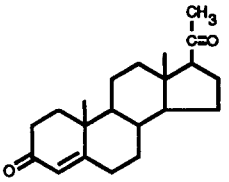
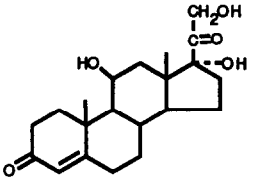
Androgens	Estrogens	Progestogens	Adrenocortical Hormones
			
Testosterone Cypionate (injectable) (Ott et al., 1950)	Urestrin; pregnant mares urine (1940) Estradiol Cypionate (Ott et al., 1950)	Progestin Sterile Solution; Porcine Corpus Luteum extract (circa 1941)	(Hydrocortisone) Adrenal Cortical extract (ACE); cattle Kuizenga, 1935) Lipo-Adrenal extract; porcine (Kuizenga, 1947)
Gonadogen; Pituitary hormones, extract of blood serum from pregnant mares (Cartland, 1934)			

Chart 2 Upjohn endocrine products in 1950. Extracts from natural sources and hormone derivatives.

Figure 2 George C. Cartland and Marvin H. Kuizenga.

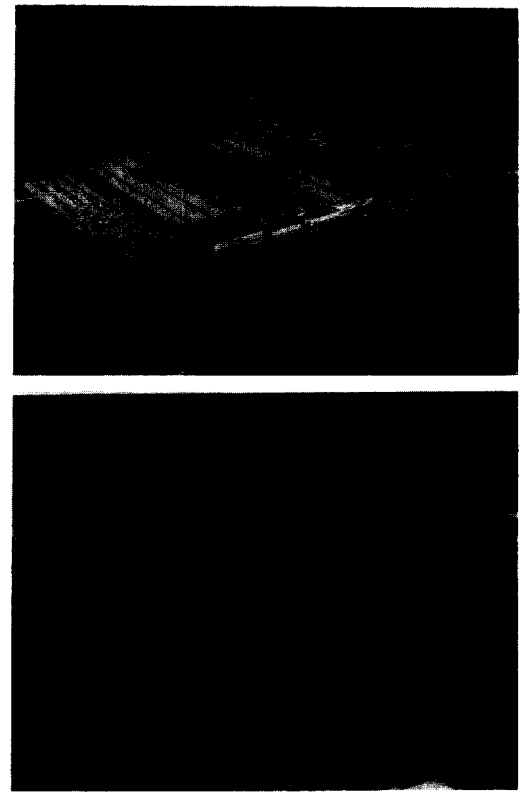


Figure 4 Manufacturing complex, 1953 and 1986.

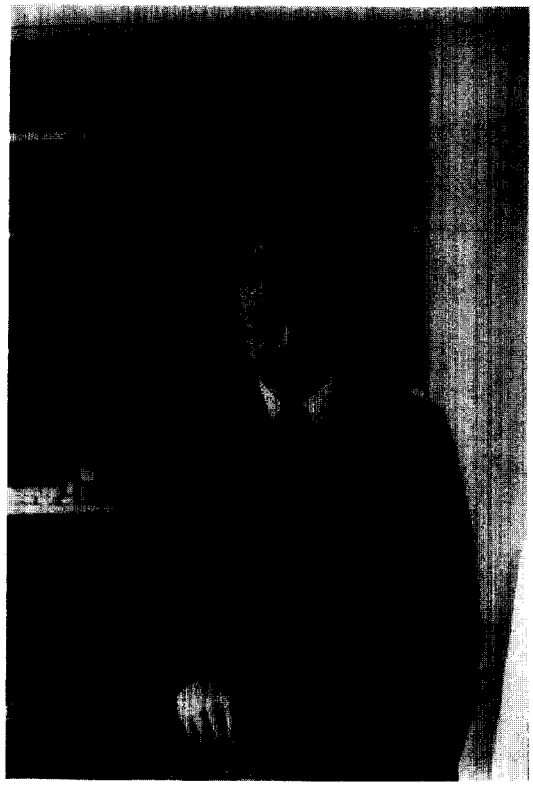


Figure 3 Donald S. Gilmore.

chemical production facility had been built for the purpose of manufacturing the vitamin folic acid via a total synthesis process devised by David I. Weisblat (a sugar chemist from Ohio State University) and his team. Fortuitously, these facilities came on stream just at the right time.

Anticipating the need for research to grow commensurate with the expectations of the new plant, Gilmore searched for someone familiar with the anatomy of a large research and development laboratory. He chose R.S. Schreiber (Figure 5) of DuPont, who arrived in January 1949, just 3 months before the April 1949 cortisone break. A polymer chemist, freely approachable and a champion of the exercise of GIF (guts, ingenuity, and faith), Schreiber's initial contribution was clearly the selection of his management team and the establishment of an effective liaison at the interface of research and development with other corporate entities, in which respect he became a timely new force.

In the period from 1945, when I came to Upjohn, to 1950, a substantial build-up of steroid chemistry research had occurred and had already reached a critical mass; four experienced steroid chemistry teams were in place, assigned to as many projects (Chart 3).

Finally, a new synthesis of progesterone[6–8] had just been devised. Work had started on progesterone in 1941 when R. H. Levin, Upjohn's first steroid chemist, came from Wisconsin to work on its synthesis from cholesterol oxidation products, but was interrupted by

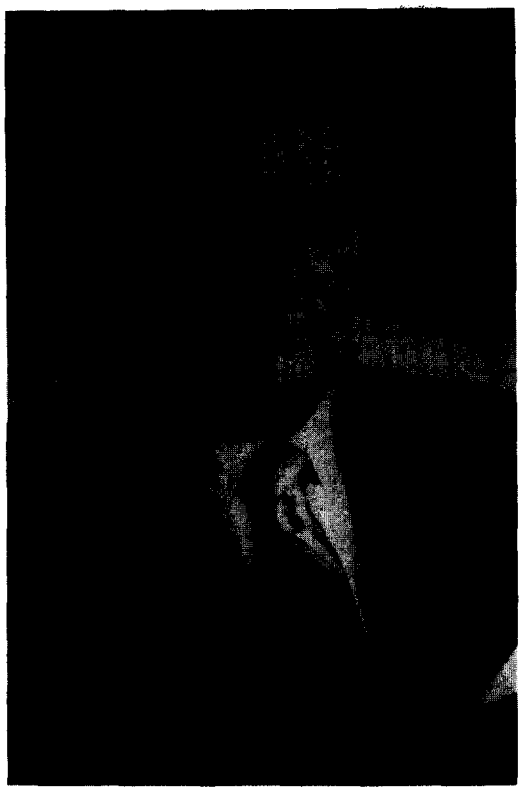


Figure 5 Richard S. Schreiber.

the war effort. The new synthesis, in the top sequence of Chart 3 (**1–5**), starts with stigmasterol, obtained from soybeans, promising a potentially inexhaustible raw material supply. Heyl, upon retirement in 1944, had gone back to the laboratory to continue the project with M. E. Herr and A. Centollela, who accomplished this transformation.

No one could have foreseen that the future role of progesterone in the synthesis of hydrocortisone would retire the remaining projects to history, let alone that the common soybean would become the major source of raw material for the synthesis of steroids. The foregoing set the stage for a highly productive research program to produce the timely cascade of steroid hormone products that ensued during the 1950s, constituting a major infusion for growth into an organization that had just set its sights on the big time.

Cortisone and beyond

Shortly after the Hench announcement in April 1949, Gilmore, already committed to the support of hormone research, met with the research groups telling us, "We want cortisone, don't spare the horses," adding to the atmosphere of excitement and accenting the question of "How?" E. G. Upjohn (Figure 6), Upjohn's first medical director, had played a strong visionary role in the evolution of the Heyl initiative for progams in the endocrine field.

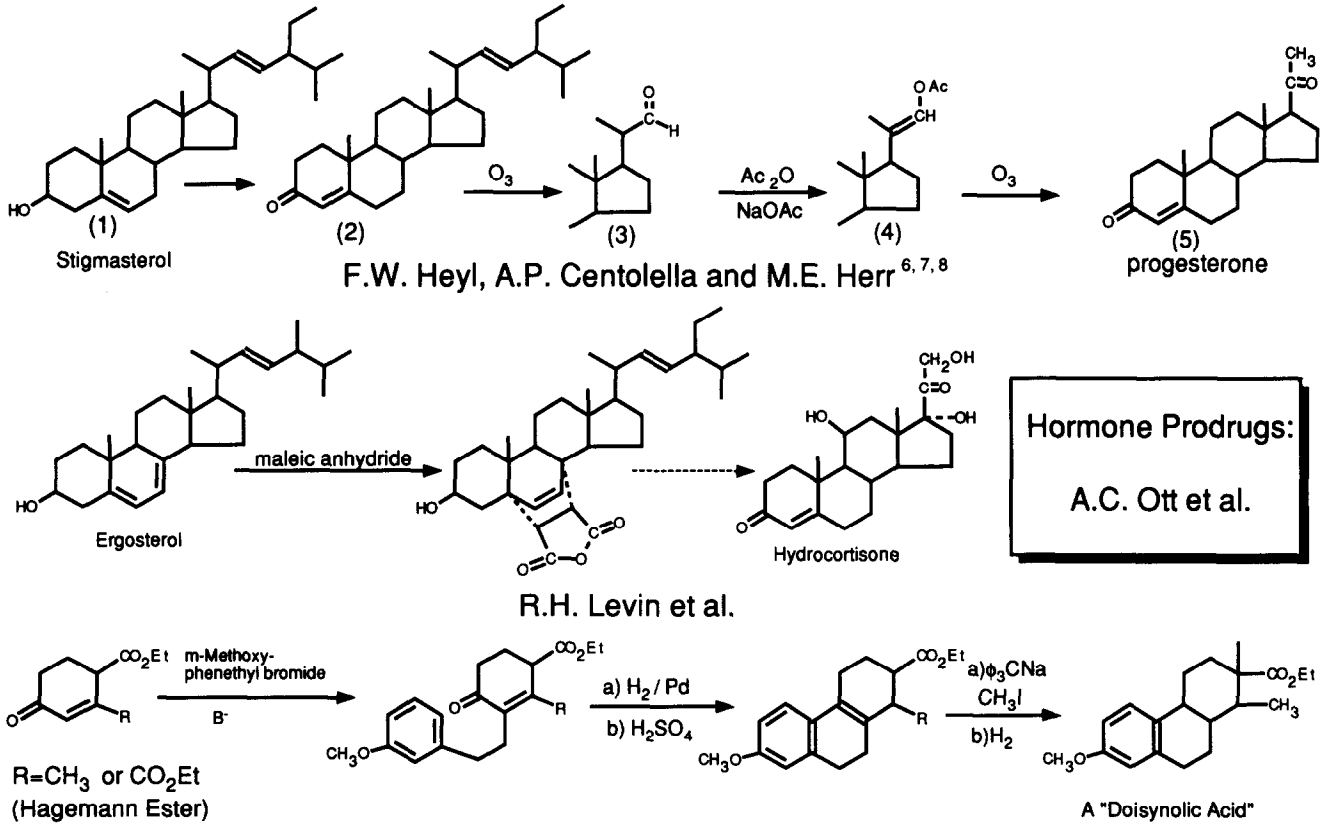


Chart 3 Upjohn steroid chemistry program in 1949 (precortisone).

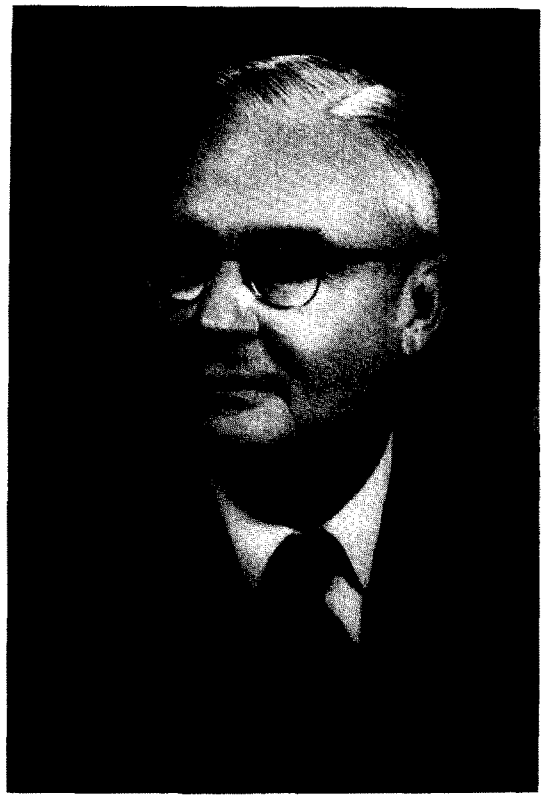


Figure 6 E. Gifford Upjohn.

The extant steroid chemistry projects continued intact, except for a few probing extensions looking at such exotic steroidlike substances as jervine and sarmentogenin, and a change of goal for the total synthesis project (Chart 4). To obtain our own cortisone, a pilot scale repeat of the bile acid process was initiated. The chemistry program comprised several independent teams working in healthy competition, each a legacy of one of the independent departmental research fiefdoms which prevailed at Upjohn at the time.

During 1949, a particularly important new dimension to this program evolved that was soon to make all else here obsolete except the progesterone work, *the enzymatic transformation of steroids*. This program comprised two competing projects (Charts 4, 4a and b) in separate departments, a healthy competition at first because of the complementary nature of their respective strategies.

It is not surprising, given Kuizenga's extensive experience with adrenal glands, that one of the competing enzymatic projects would be the investigation of adrenal gland homogenates for the direct enzymatic 11β-hydroxylation of selected steroid precursors (Chart 4, 4b), which was initiated in the endocrinology department under W. J. Haines.

The genesis of the other enzymatic project (Chart 4, 4a), the screening of several selected steroid substrates

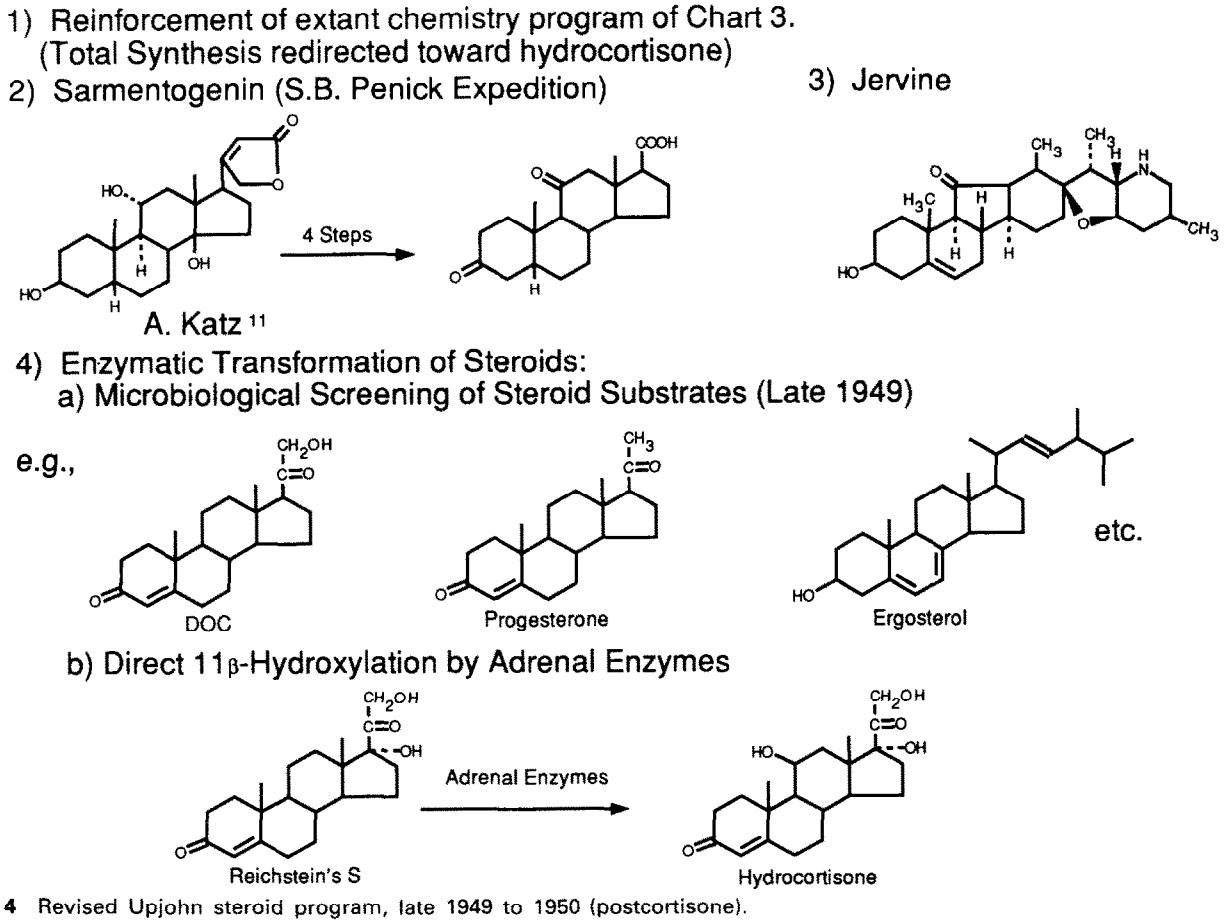


Chart 4 Revised Upjohn steroid program, late 1949 to 1950 (postcortisone).

with microorganisms looking for direct hydroxylations or any useful transformations, is clouded by myth and counterclaim. The idea for such an approach had occurred independently to several, possibly to some even by subliminal assimilation. The single advocacy story that has been perpetuated is not correct. D. H. Peterson in his autobiography (1985), Steroids 45:1–17, makes such a claim while neglecting to clarify the central role of his colleague H. C. Murray. But the misrepresentation of Peterson's role first gained quotable status much earlier in the classical text on steroids by Fieser and Fieser (1959) published by Reinhold, page 673. "Peterson and his group" were credited with the discovery—apparently on the basis of authorship on the full paper (Ref. 5) which appeared after an earlier announcement (Ref. 3) in the form of a communication to the editor. Curiously this earlier communication, authored by Peterson and Murray only, was not cited. Peterson was not the leader of a group in 1950 when the discovery was made.

The fact is that Murray and Peterson made this discovery jointly as documented in ref. 3. Both were awarded the Upjohn prize in 1951 for their seminal discovery and they are listed as co-inventors on U.S. Patent 2,602,769, "Oxygenation of Steroids by Mucorales Fungi," granted in 1952. A review of the pre-1950 scenario, as follows, provides insight into the question of priority for initiative.

In his first organization change later in 1949, Schreiber had brought in the right person to head chemistry. In a move that exhibited his combination of vision, insight, and leadership, D. I. Weisblat (Figure 7), in his new role, engineered the assembly of an interdisciplinary team in chemistry under R. H. Levin, combining steroid chemists, biochemists, and microbiologists to investigate the microbiological approach. This project was unique at Upjohn; such collaboration between interdependent disciplines was not only healthy, it was essential.

The genesis of this microbial project had begun to evolve earlier. Of relevance is that H. C. Murray had joined Upjohn in 1942 in bacteriology research to work on the production of riboflavin by fungi; in 1946 he was recruited into the nutrition department by Weisblat to investigate among other things *the use of microorganisms to produce intermediates for chemical synthesis.* In this same year, D. H. Peterson, trained in endocrine biochemistry under F. C. Koch at the University of Chicago and coinventor-to-be with Murray of the microbiological 11α-hydroxylation, came to Upjohn to work in antibiotics research.

In fact, timely publications in 1949 provided ample cues that enzymes of both microbial and mammalian origin have the potential for useful transformations of steroids. This included the report of Kra'mli and Horvath[12] in early 1949 describing the 7-hydroxylation of cholesterol by *Proactinomyces roseus,* which, according to Murray, was called to his attention by Weisblat, suggesting that Murray "pay attention" to such an approach; in the same year, Hechter et al.[13] at the Worcester Foundation reported the direct 11β-hydroxylation of 11-deoxycorticosterone by adrenal gland perfusion.

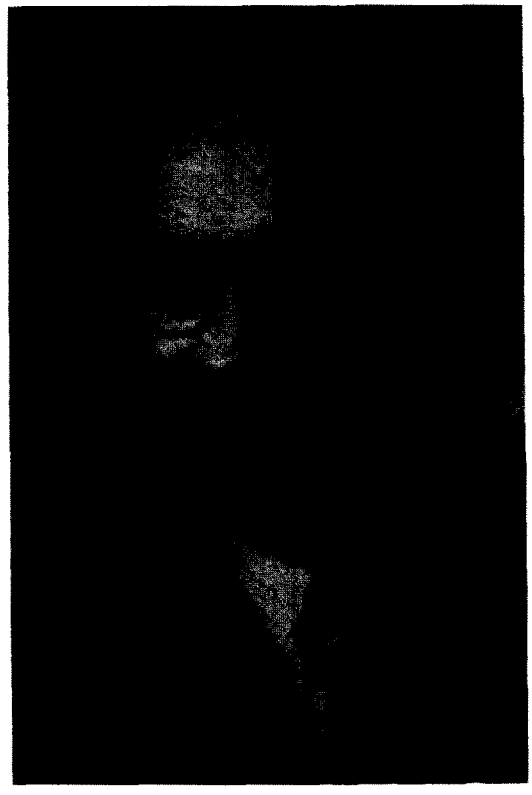


Figure 7 David I. Weisblat.

The new interdisciplinary team came together in November 1949. Peterson, who frequently played handball with Weisblat and became an outspoken advocate, and his assistant Lester Reineke, experienced in paper chromatography, were transferred to Levin's group in the chemistry department to join microbiologist Murray and others, forming the core of the microbiological project. After this, S. H. Eppstein and A. Weintraub, having already initiated a similar project in September 1949 in the bacteriology department with J. H. Edwards, were transferred to chemistry, combining common research interests to focus on the microbiological approach.

According to Levin (Figure 8), "There was never a flash of genius; the strategy was evolved over time by the group working together." Basically, the strategy evolved was to speed the process of screening a large number of microorganisms by working on a microscale and quickly determining by paper chromatography whether alteration of the selected steroid substrates had occurred. Scale-up and isolation studies were limited to screen results that indicated substantial levels of only a few conversion products. It was of necessity a cooperative venture. Precisely at that time, Zaffaroni et al.[14] published paper chromatography procedures developed specifically for identifying the adrenal steroids. Levin dispatched Peterson and Eppstein to visit Zaffaroni. This timely publication, after some adapta-

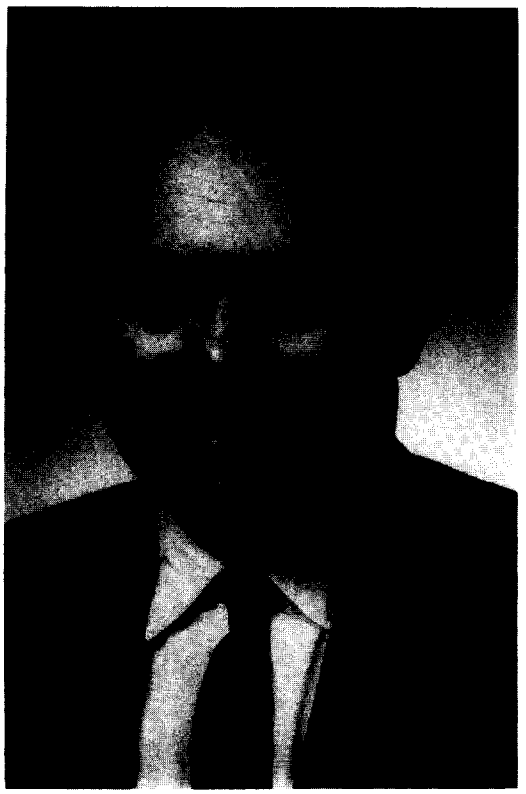


Figure 8 Robert H. Levin.

tion, did indeed step up the timetable for initiating the planned screening strategy, but it was the luck of the draw that preempted the anticipated longer term demands of such a screen.

Progesterone was selected as one of the substrates for obvious reasons, not the least of which was the new synthesis at Upjohn from the abundant sterol, stigmasterol. Included in Murray's strategy of selection from a broad spectrum of microorganisms was the well-known technique of intentionally exposing agar plates to obtain cultures of organisms in the environment, in this case seeking the most common "wild" organisms. In February 1950 Murray found that progesterone, fermented with one such mold on a microscale, was converted cleanly into two products as shown by papergram. This was repeated on a larger scale, extracted, and the crude extract turned over to Peterson for isolation and characterization of the products.

Durey Peterson isolated two crystalline products. One of these crystalline substances turned out to be 11α-hydroxyprogesterone, as proved by oxidation to the known 11-ketoprogesterone (Chart 5) obtained from T. F. Gallagher of Sloan-Kettering. The other proved to be a secondary hydroxylation of 11α-hydroxyprogesterone at carbon-6, i.e., 6α-11α-dihydroxyprogesterone. This remarkable accomplishment was completed before mid-1950.

Murray is fond of noting that had he used a more

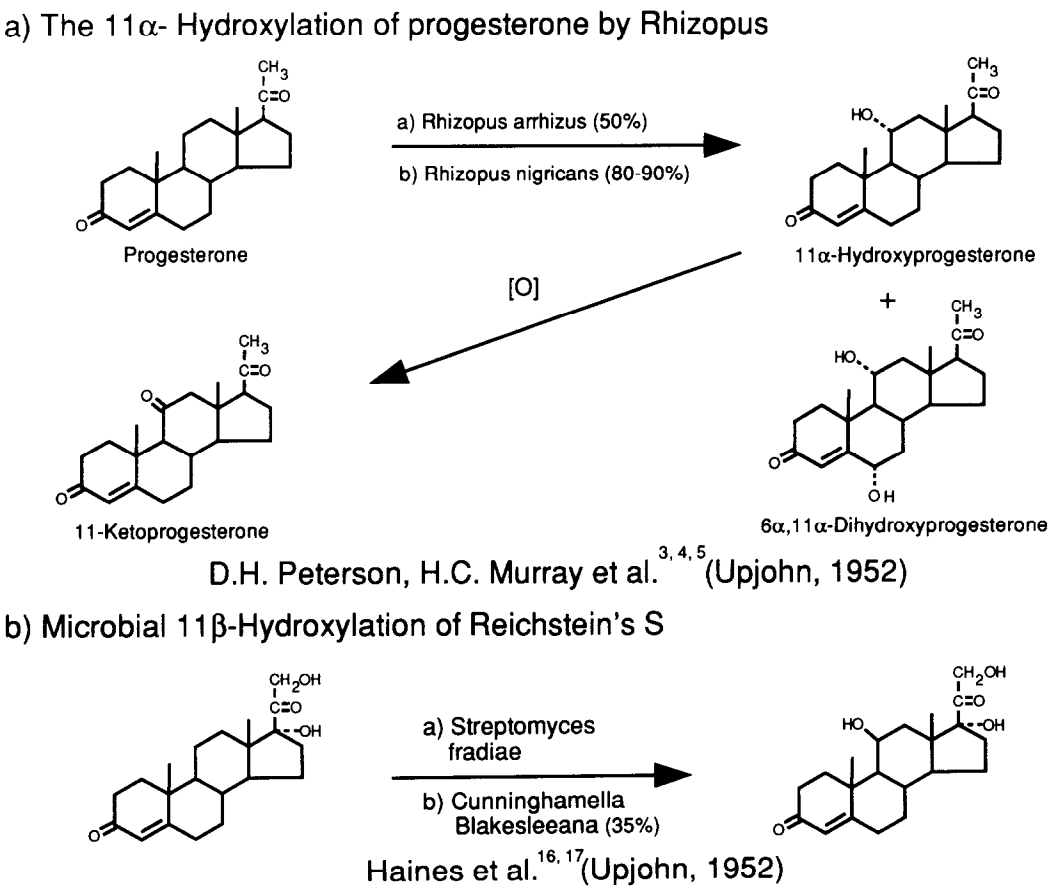


Chart 5 Enzymatic oxygenation of steroids at carbon-11.

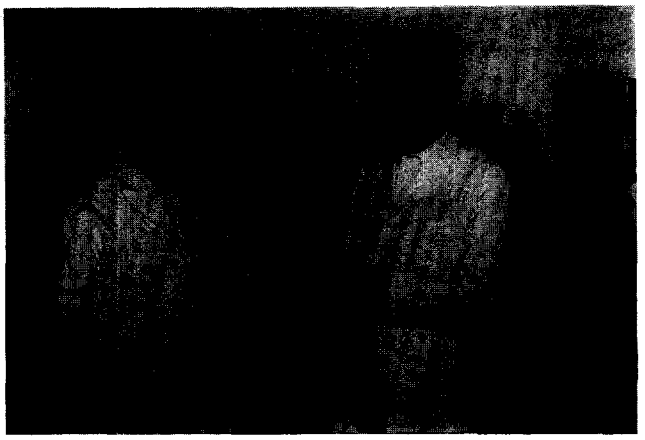


Figure 9 Herbert C. Murray and Durey H. Peterson.

efficient shaker for aeration, this historic discovery would have been put off for some time, since rapid stirring was later shown to produce only the dihydroxyprogesterone.

Myth is often the classical fate of scientific discovery that comes to public notice; the facts become clouded by romantic or false interpretation. Writers, seeming to insist on analogy with the story of the Fleming penicillin discovery, have fabricated the myth that the *Rhizopus*/steroid discovery was an accident; somehow on a window sill of an old tin shed an agar plate just happened to produce this unexpected result, or in one instance that a watermelon rind was the stray *Rhizopus* host. Even a phantom third inventor, "H. Corners," emerged—an amusing misinterpretation of Murray's address, which is Hickory Corners.

Within the framework of a disciplined and well-coordinated project Murray and Peterson (Figure 9), individuals of sharply contrasting nature, had made a history-making discovery of the unobvious, the hallmark of invention by definition and hailed as "the neatest trick of the year."[15]

Murray, a professional committed to the art and science of microbiology, modestly but freely recalls the complex interactions and technical detail of the time in remarkable detail. Biochemist Peterson was characteristically single-minded and zealous. Ironically, in his last years he suffered from the severe pain of spinal arthritis which did not respond well to corticosteroids.

After this astonishing breakthrough, serendipity played a role in the Haines et al. project (Chart 5b) for the conversion of Reichstein's substance-S to hydrocortisone.[16,17] In short, a better than expected yield of hydrocortisone from an adrenal enzyme preparation was found to be due to contamination with *Streptomyces fradiae*. After further screening, *Cunninghamella Blakesleana,* a fungus of the Mucorales order, was then found that yielded hydrocortisone, initially in 35% yield, and then later improved. Superficially this exciting microbiological transformation would appear to be of greater importance than the first above. Now microbial enzymes had achieved the ultimate goal, that of simulating mammalian enzymes exactly by introducing oxygen into the 11-position in the natural 11β-configuration, requiring no further chemical manipulation.

Upjohn would have to make a choice. With these two competing microbial 11-oxygenations in hand, one based on the 11α-hydroxylation of progesterone and the other on the direct 11β-hydroxylation of Reichstein's substance-S, the baton was passed to the chemistry groups.

An obvious prize would be a "direct" synthesis of hydrocortisone from 11α-hydroxyprogesterone, i.e., a synthesis in which ring A remains intact in the process (Chart 6). Such a process would also likely be applicable to the synthesis of Reichstein's S directly from progesterone should 11β-hydroxylation prove to be the preferred enzymatic route.

The disadvantage of an alternate "indirect" route from 11α-hydroxyprogesterone (**2** to **3** to **5**) was the cost of eliminating and then reconstituting the ring A structure. The advantage was the certainty of meeting the demand for cortisone, and it was still possible that other structures, such as **6,** would become superior microbiological substrates. Both strategies were addressed, along with renewed emphasis on the production of progesterone from stigmasterol (Chart 7).

Substantial improvements in the Herr/Heyl/Centollela process for conversion of stigmasterol (**2**) to progesterone (**7**) were made in due course.[19–21] Milton Herr[19] developed the highly selective reaction of piperidine with 3-ketobisnor-4-cholenaldehyde (**5**) to form the enamine **6,** which in turn reacts selectively with ozone (or $Na_2Cr_2O_7$) to yield progesterone. A synthesis from ergosterol (**3**)[20] was integrated into this process. Upjohn was to become completely independent of any other source of progesterone.

But Russell Marker's pioneering work at Penn State on the isolation and structure of plant sapogenins and the synthesis of progesterone from one of them, diosgenin (**4–7**),[22] had been consummated commercially earlier as a result of his much publicized and complex Mexico/Syntex venture. As a strategy to speed up the timetable for cortisone production, Upjohn purchased substantial quantities of progesterone to bridge the gap until our own progesterone process came on stream. This was Mr. Gilmore's decision, despite conflicting advice, and he personally negotiated the purchase.

(On a personal note, it was Marker's work that inspired my interest in steroids. As a chemistry student at Penn State in 1942, I had asked Dean Frank Whitmore about the possibility of working with Marker, and was told without explanation that "Professor Marker would soon be closing out his laboratory." Of course, his Mexican venture soon became public knowledge.)

Shortly, Upjohn's J. Ward Greiner, a chemical engineer, began work part-time on his own initiative in the pilot laboratory to develop counter-current distribution techniques to separate stigmasterol from sitosterol,[18] as noted in Chart 7, upper left. According to Greiner, he was encouraged by Schreiber's general policy back in research on the use of "10% free time," and specifically on this project by Weisblat, who characteristically

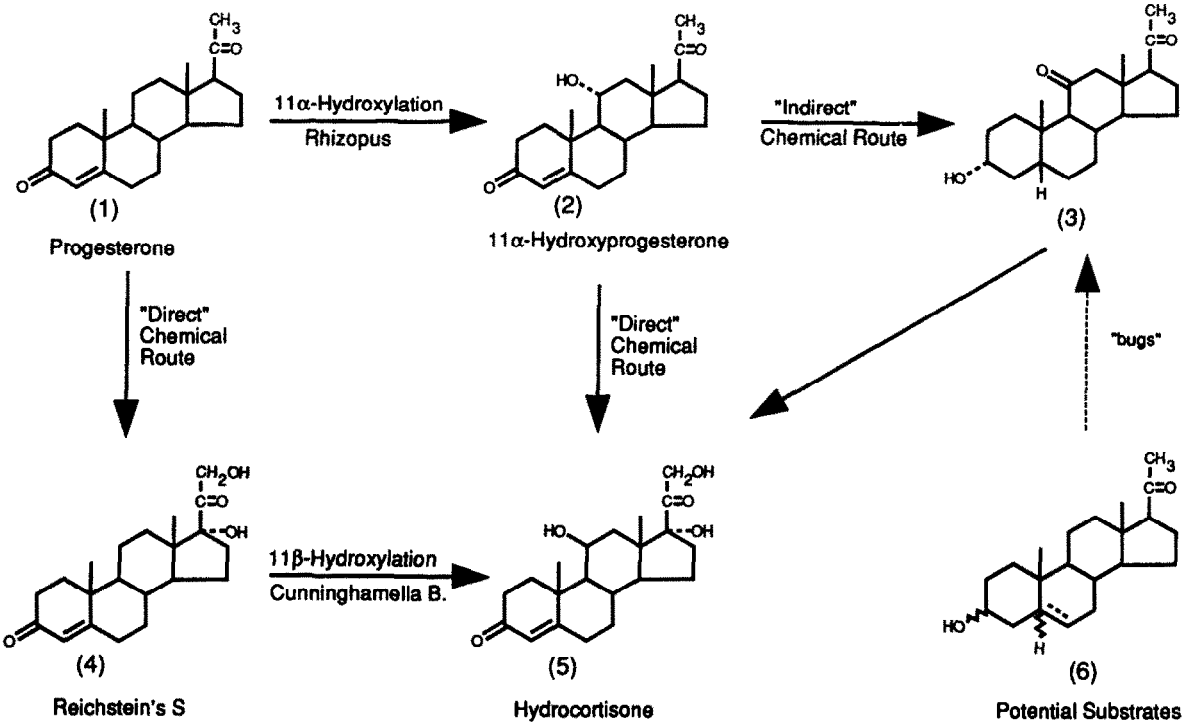


Chart 6 Strategy: combined chemical/microbiological process.

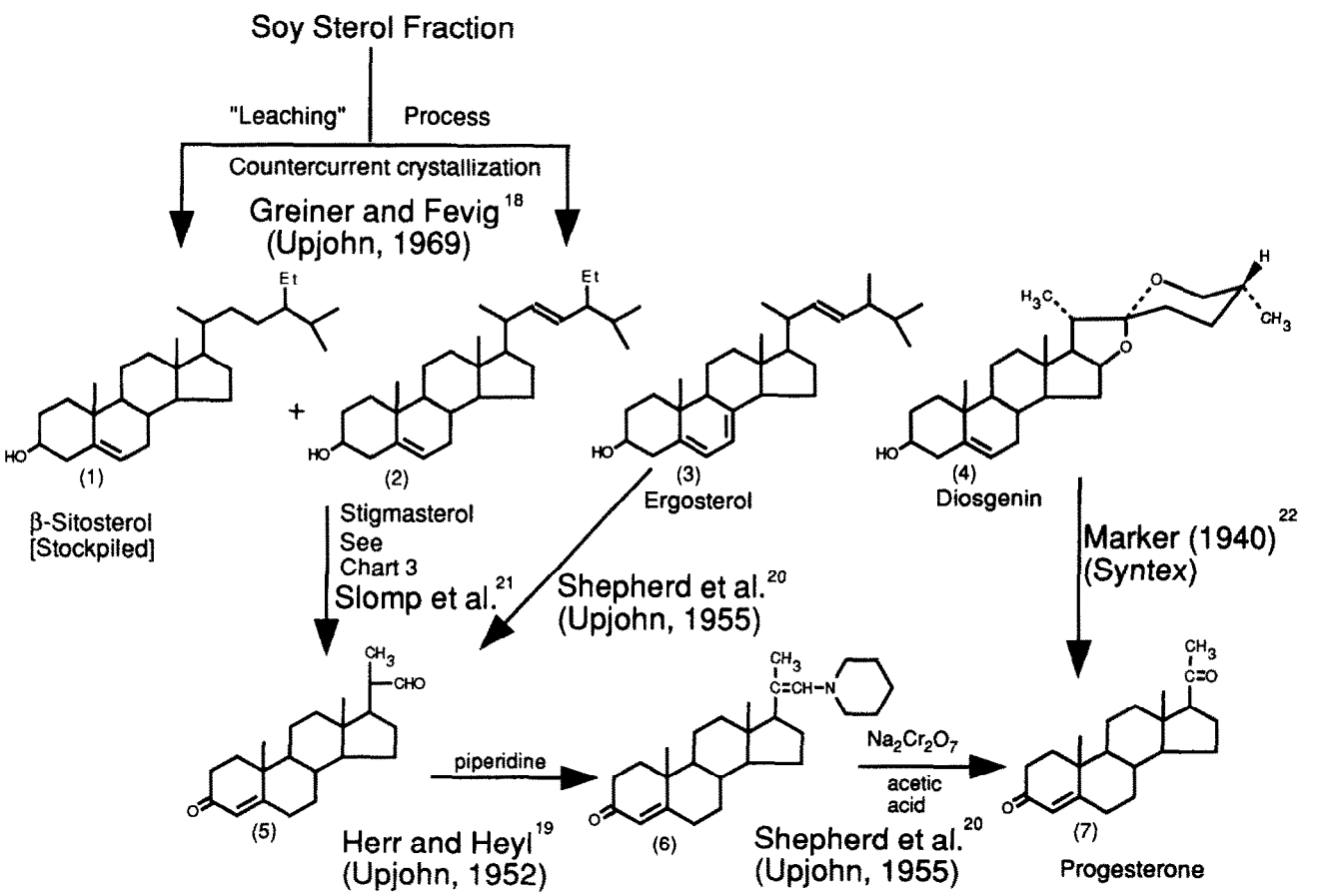


Chart 7 Progesterone synthesis from major plant sterols.

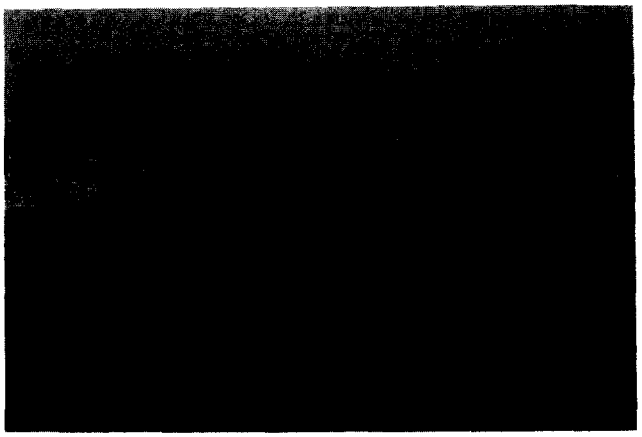


Figure 10 Sistosterol stockpile.

became enthusiastic and influential in matters beyond his direct responsibility.

The preview photo of the discarded sitosterol stockpile in Figure 10 graphically demonstrates that he succeeded, and it dramatizes 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. This was to be realized in due time by another history-making development, to be discussed later, which matched that stemming from its sister sterol stigmasterol via the microbiological oxidation of progesterone.

The first synthesis of hydrocortisone from 11α-hydroxyprogesterone was devised at Upjohn by the "indirect" strategy as shown in Scheme 8.

Ring A was first saturated by the novel sequential reductions shown (**1–3**)[23,24] to produce the desired pregnane-3α-11α-diol-20-one (**3**, top sequence).

It is here that a new and unexpected dimension in the competitive equation surfaced: the policy of exalting first publication over proprietary considerations. In this case, Syntex, anticipating the next steps of the more proprietary-minded Upjohn based on emerging disclosures, published this same two-step sequence from 11α-hydroxyprogesterone in 1952.[25] In this publication they also reported confirmation of the Upjohn microbiological synthesis[3,4] of 11α-hydroxyprogesterone from progesterone by a fungus of the *Rhizopus* genus, which they obtained from a Mexican soil. They then claimed a formal 10-step synthesis of hydrocortisone from progesterone, because the 11-keto compound corresponding to **3** (Chart 8) had already been converted to hydrocortisone by Kritchevsky et al.[26,27] However, their source of 11α-hydroxyprogesterone for

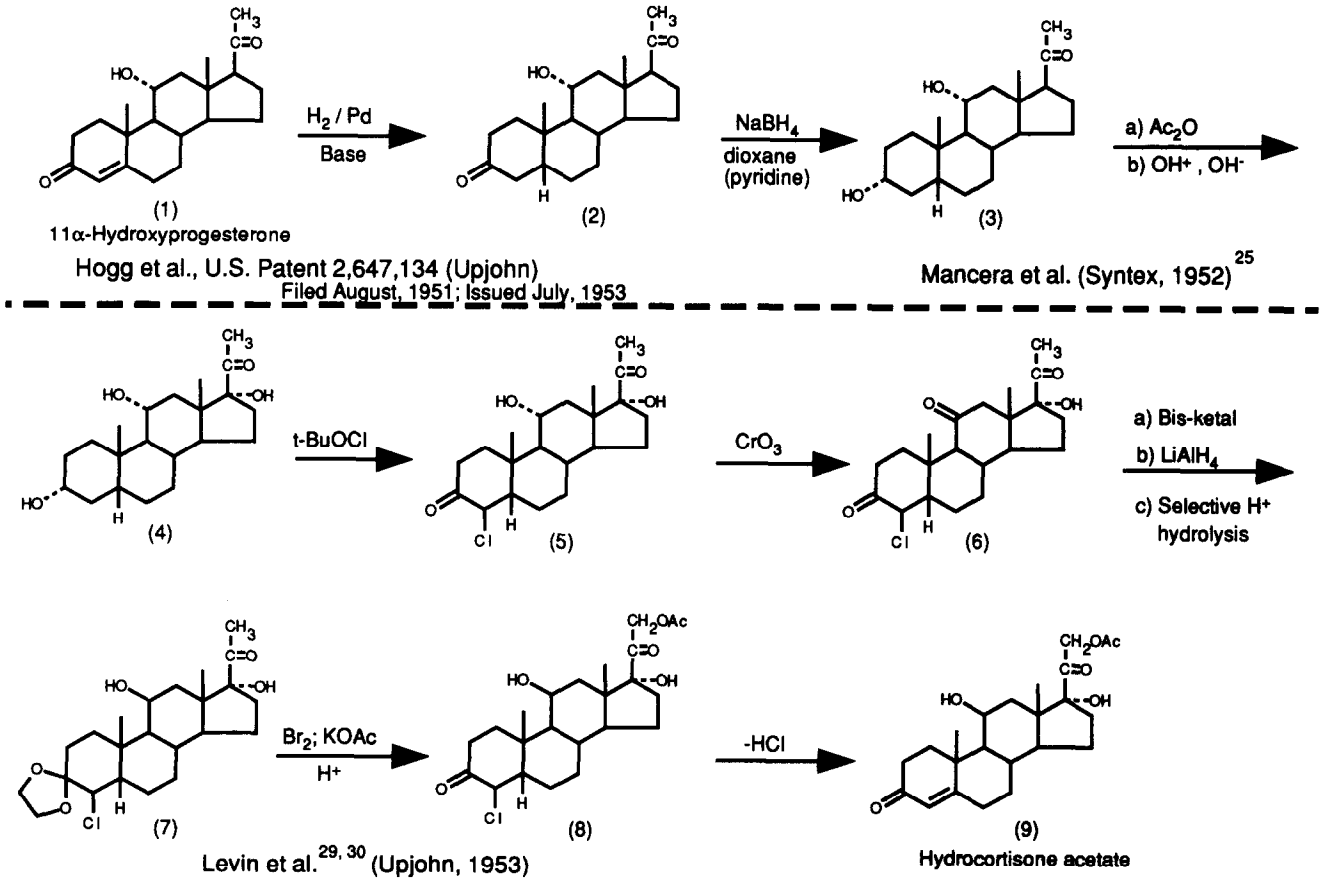


Chart 8 Upjohn indirect route to hydrocortisone from 11α-hydroxyprogesterone.

this work was implied to be otherwise by reference to unpublished work on its synthesis from diosgenin.

In these most tenuous of circumstances, priority is not even a moot point, because these reductions had been disclosed in an Upjohn U.S. Patent[23] issued in 1952, but filed in August of 1951, which is also the date that the Upjohn patent application on the 11α-hydroxylation of progesterone was published in the *South Africa Journal of Patents* and became available to the public upon request.[28] Further, Levin's group had already completed the synthesis of hydrocortisone by new chemistry,[29,30] shown below the dotted line (Chart 8, **4–9**), and Upjohn was in first production of cortisone by June 1952, and then hydrocortisone in 1953. But even this process was to become an interim production strategy.

Concurrently, attempts to devise a "direct" route from 11α-hydroxyprogesterone were underway (Chart 9). The strategy was to attempt selective base–catalyzed acylation of the 21-methyl group of 11-ketoprogesterone to activate it for further reactions. Using diethyloxalate, it was found very early by A. H. Nathan and F. H. Lincoln that 21-monoacylation was indeed the predominant product, as predicted, but apparently it was always accompanied by some 2,21-bisglyoxalate. The first studies were conducted on the presumed 21-monoglyoxalate for proof, and to establish the feasibility of the strategy (Chart 10).[31]

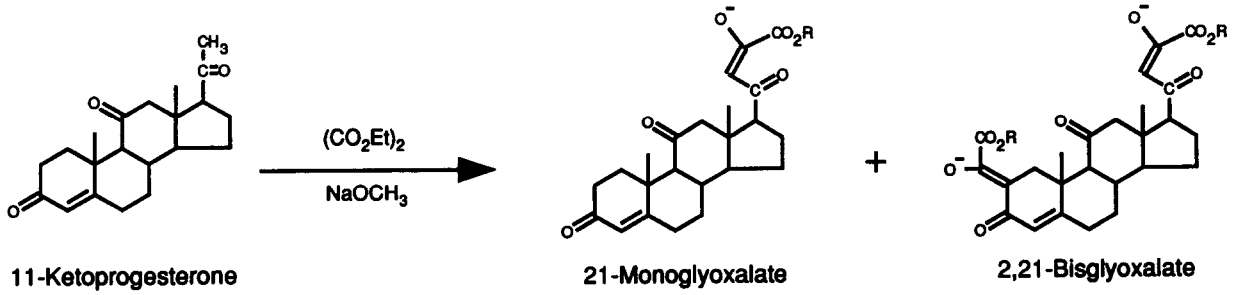


Chart 9 Upjohn direct route to hydrocortisone. Initial strategy: selective 21-acylation.

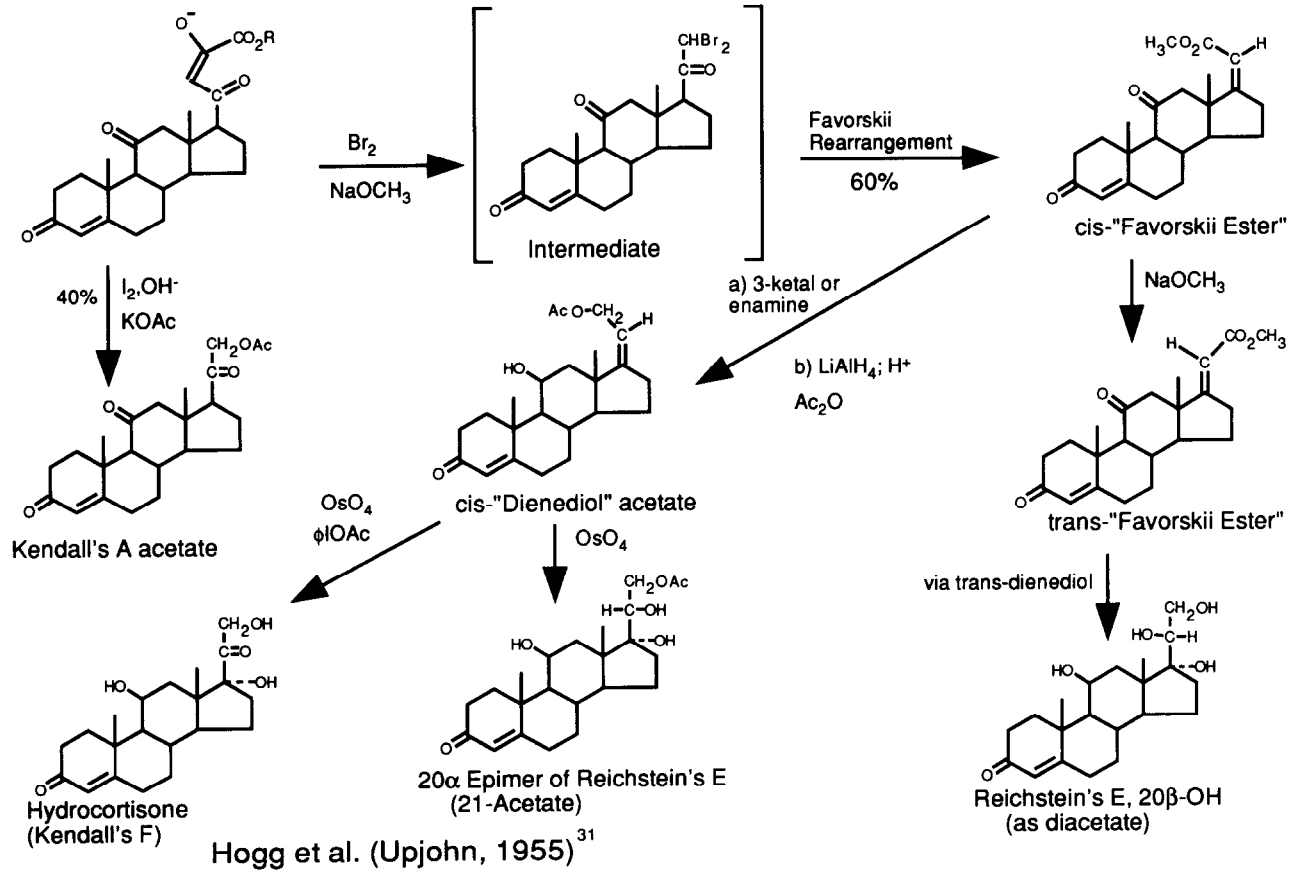


Chart 10 Upjohn direct route to hydrocortisone, via 11-ketoprogesterone 21-monoglyoxalate.

The proof of preferential 21-monoglyoxalation came from its conversion to Kendall's compound A (directly below) in 40% yield by the method of Ruschig.[32] This is the same corticoid supplied by Merck for the first but unsuccessful trials by Hench in rheumatoid arthritis.

Then one of the envisioned applications of the 21-monoglyoxalate [dibromination, alkaline cleavage of glyoxalate, and Favorskii rearrangement (Chart 11)], spearheaded by P. F. Beal, yielded the *cis*-"Favorskii ester" (as we later called it), which he then used to produce the first few milligrams of hydrocortisone via *cis*-dienediol acetate, as shown.

That the Favorskii ester is *cis* was first suggested by its base-catalyzed inversion to a more stable form, presumably *trans*. Unequivocal proof of the assigned *cis* and *trans* stereochemistry came from their conversions, as shown (Chart 10), to the adrenal substances of known configuration, the 20α and 20β epimers of Reichstein's E, respectively.

However, the scheme that was to become Upjohn's main-line process was a variation based on the intentional overglyoxylation of 11-keto-progesterone with two or more moles of diethyl oxalate (Chart 11).[33]

The strategy was to use the 2, 21-bisglyoxalate (**1**) in the same Favorskii sequence as before, anticipating that all substitutions at the 2-position in ring A would be reversible, leaving ring A intact.

Lincoln and Nathan demonstrated that the Favorskii sequence (Chart 11, **1–3**) does indeed yield the predicted 2-bromo Favorskii ester (**3**), which is usually not isolated but directly debrominated with zinc to Favorskii ester (**4**). They then defined optimum conditions with significant improvement in yield.

The proof that bromine was in the 2-position was obtained from spectral analysis of the dehydrobromination product, 1-dehydro Favorskii ester (**8**), which turned out, serendipitously, to be a precursor to the important but as yet unborn corticosteroid analog, 1-dehydro hydrocortisone (prednisolone).[36,37]

The Favorskii ester (**4**), thus obtained from **3**, was converted to hydrocortisone via the revised procedure, intermediates **4–7** of Chart 11. This process was rigorously developed by an elite team now including B. J. Magerlein, W. P. Schneider, and others[31,33] thus completing the preferred direct route to hydrocortisone.

Note that, in the $LiAlH_4$ reduction of the enamine **5**, both the 11-keto group and the 21 ester are reduced concurrently so that now the original 11α-hydroxyl has been inverted to the correct 11β configuration. Isolation of intermediates became necessary only at the Favorskii ester and the dienediol acetate stages.

In those days at Upjohn the definition of optimal laboratory procedures was the responsibility of the originators. The subsequent translation and scale-up to

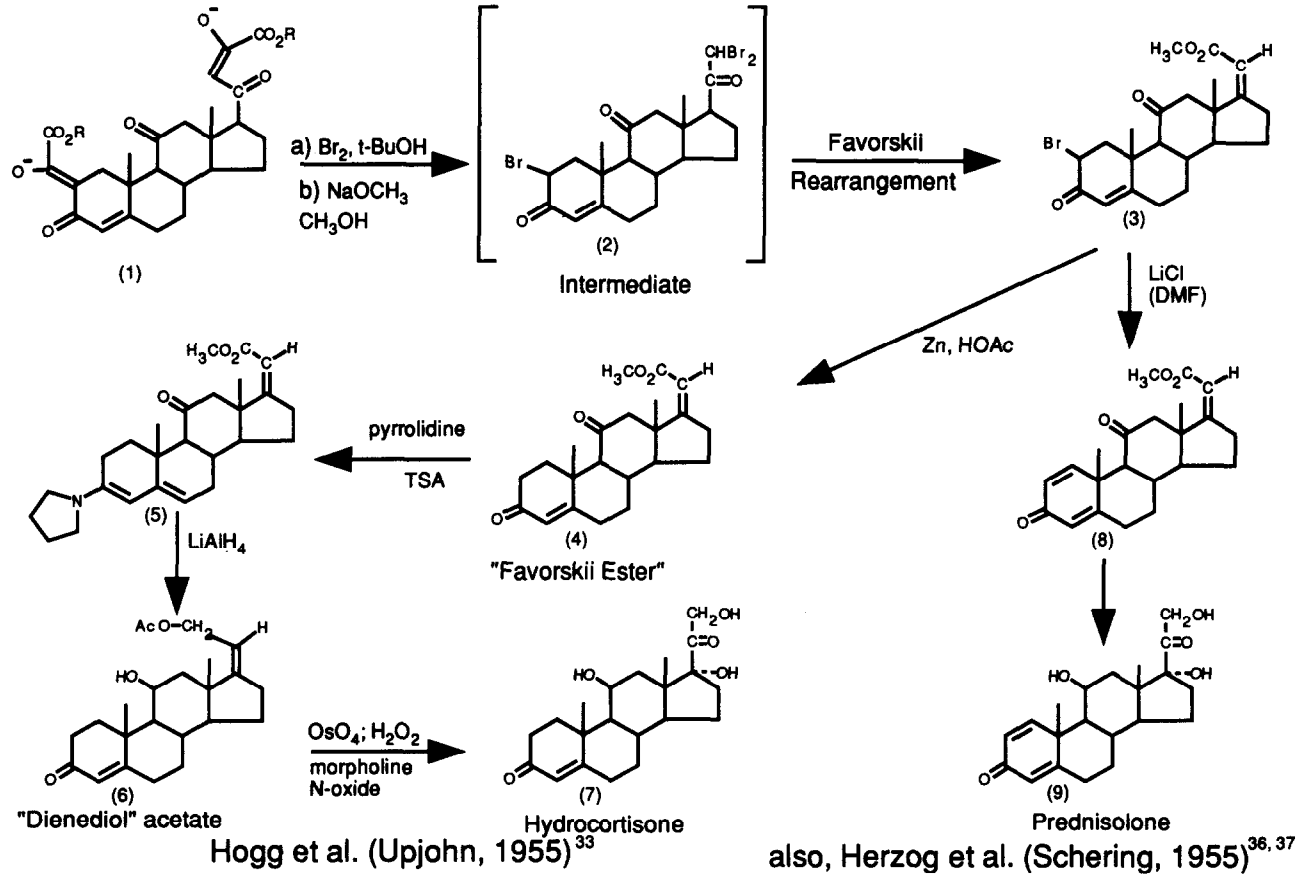


Chart 11 Upjohn direct route to hydrocortisone, via 11-ketoprogesterone 2,21-bisglyoxalate.

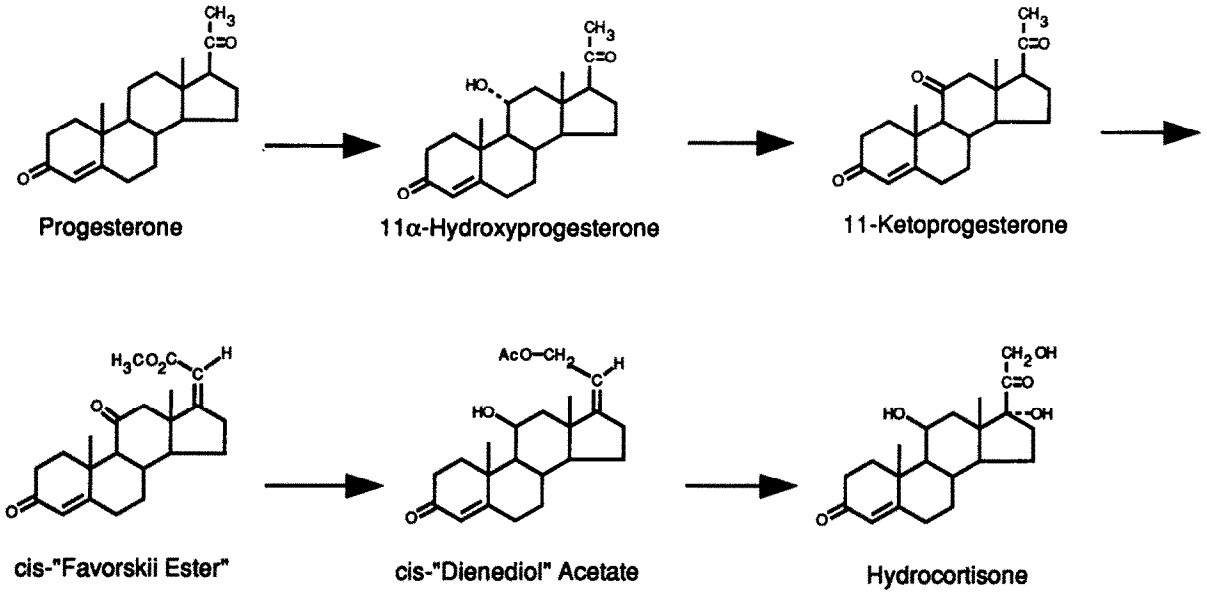


Chart 12 Upjohn direct route to hydrocortisone. Main-line process, condensed.

industrial levels, especially of the complex and difficult Favorskii sequence, was an admirable accomplishment led by Donald R. Myers and H. Alden Drake.

The direct process, condensed as on Chart 12, completes an essentially five-step combined microbiological/chemical process for the synthesis of hydrocortisone from progesterone. The direct route was published in part in the form of two communications,[31,33] but only after some time. We had been told "maybe never."

Yields in the progesterone microbial oxidation reached 90% with *Rhizopus nigricans*, whereas the 11β-hydroxylation of substance-S, a viable contender for some time, encountered scale-up problems. The need for contingency processes to support the latter alternative disappeared.

The potential for overall yield also ensured entrenchment of this new direct process, as did the foreseen potential for steroid analog synthesis from the novel and exclusive intermediates.

In 1952, after another of Schreiber's organizational changes, the steroid chemistry groups at Upjohn were consolidated, becoming my responsibility. This allowed us to launch the aforementioned concerted effort to develop the new chemical process and to accelerate the modest beginning of the corticosteroid analog synthesis program.

The use of these exclusive and versatile intermediates for analog synthesis was at first an expediency. Then it became a deliberate strategy of process integration with ultimate economy in mind, which later helped Upjohn to become the leading producer of the corticosteroids.

This direct process remains in use after nearly 40 years without change in basic strategy, but much improved over time due to the innovative contributions of a subsequently constituted process research and development group.

Steroid hormone analogs

In 1953, Fried and Sabo[34,35] announced the synthesis of the first highly potent steroid analog, 9α-fluoro hydrocortisone, formally accenting the beginning of the analog era.

Shown here (Chart 13), in prevue, are the most important single substituents and their locations on the hydrocortisone molecule discovered by the steroid community that either enhance antiinflammatory activity, override unwanted therapeutic effects, or both.

Distilled out of an enormous effort in the entire steroid community, these few substituents became the building blocks of most of the important therapeutically useful cortical analogs. They are dehydrogenation at position 1; halogen at positions 9 and 16; methyl at positions 2, 6, and 16; and 16α-hydroxyl and 21-desoxy. As we will see, many of these discoveries arose from serendipity and empiricism more than one may like to think, further demonstrating the wisdom of Pasteur's axiom, "Chance favors the prepared mind."

The contribution of each to the consequent therapeutic profiles when introduced into the hydrocortisone molecule in pairs, triplets, or quadruplets became increasingly predictable as the data base grew, and thus more competitive as others followed the leaders. It is impressive that all of these key locations (2, 6, 9, 16, and 21) are in juxtaposition to the sites of metabolic inactivation of the hydrocortisone molecule. For a comprehensive review of corticosteroid analogs and their biological and medical properties, see Sarett et al.[38])

The next few Charts (14–17) record (a) The discov-

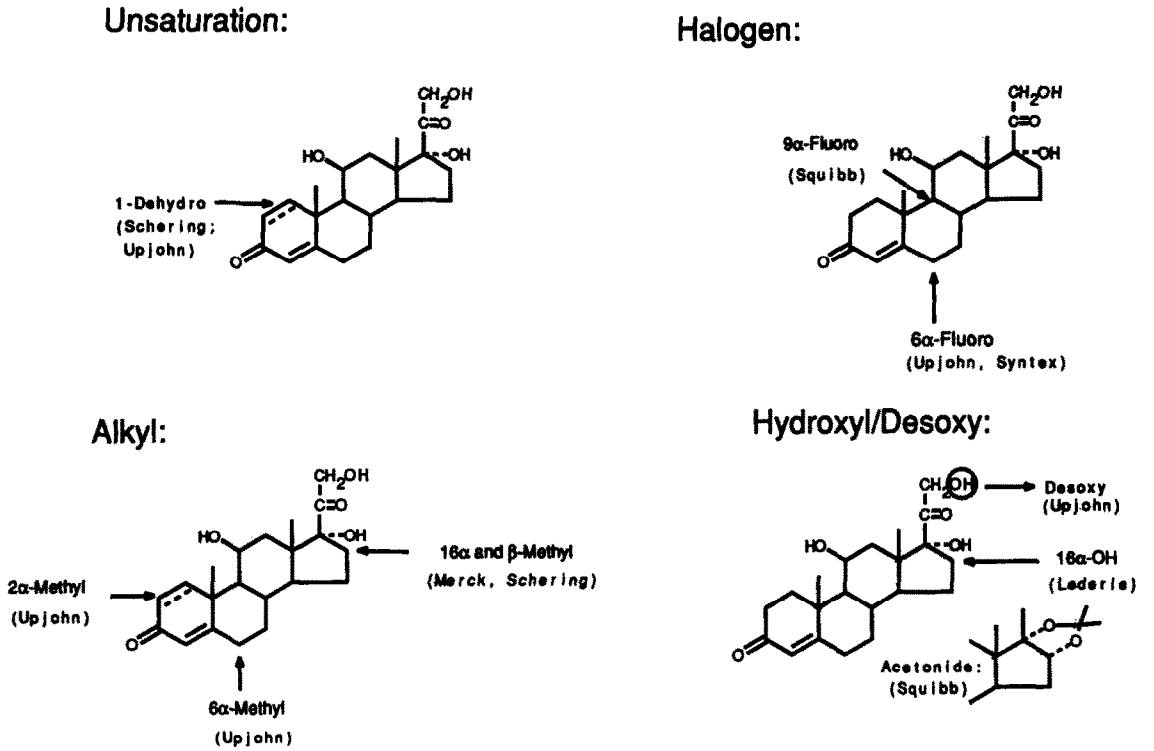


Chart 13 Corticosteroid analogs: preview of corticoid activity modifying discoveries.

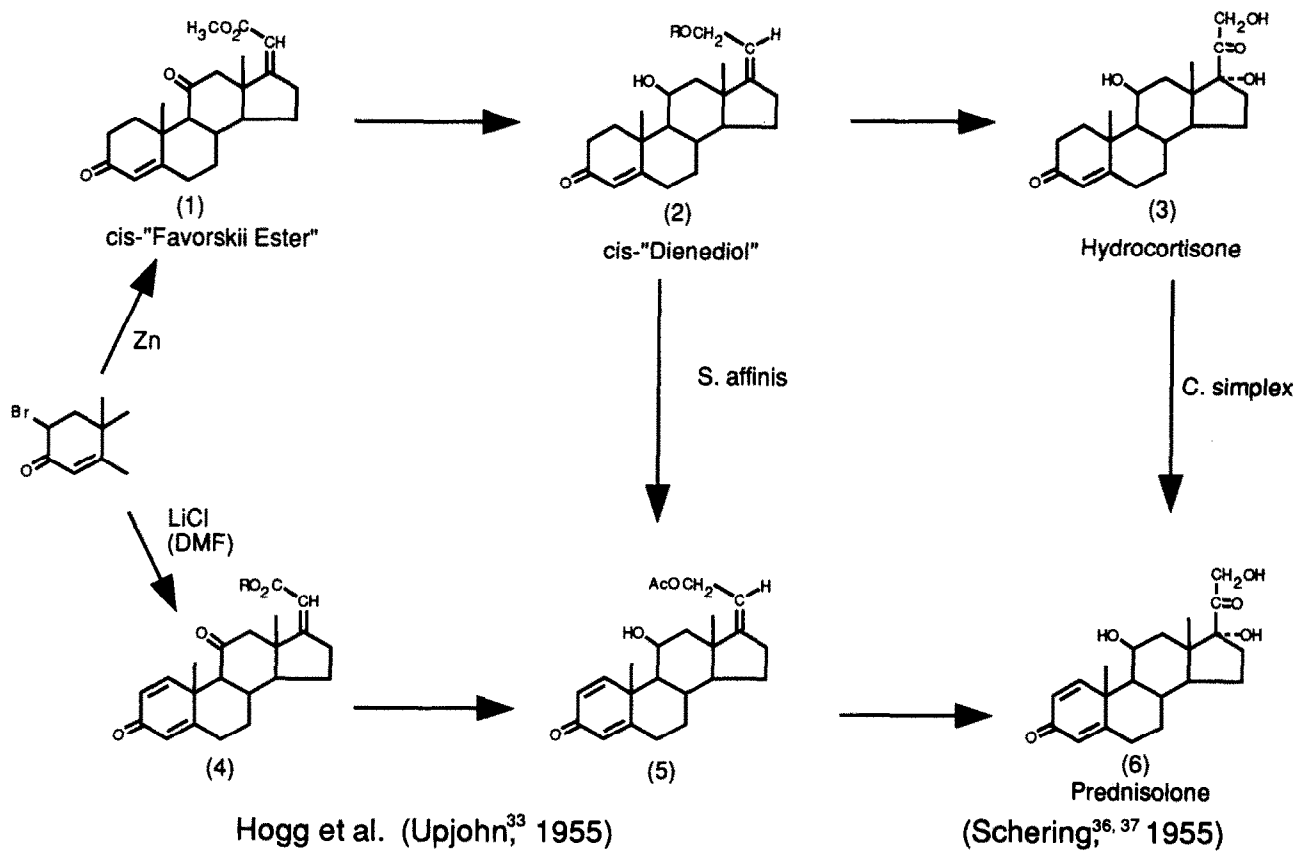


Chart 14 1-Dehydro analogs: discovery and synthesis from Upjohn main-line intermediates.

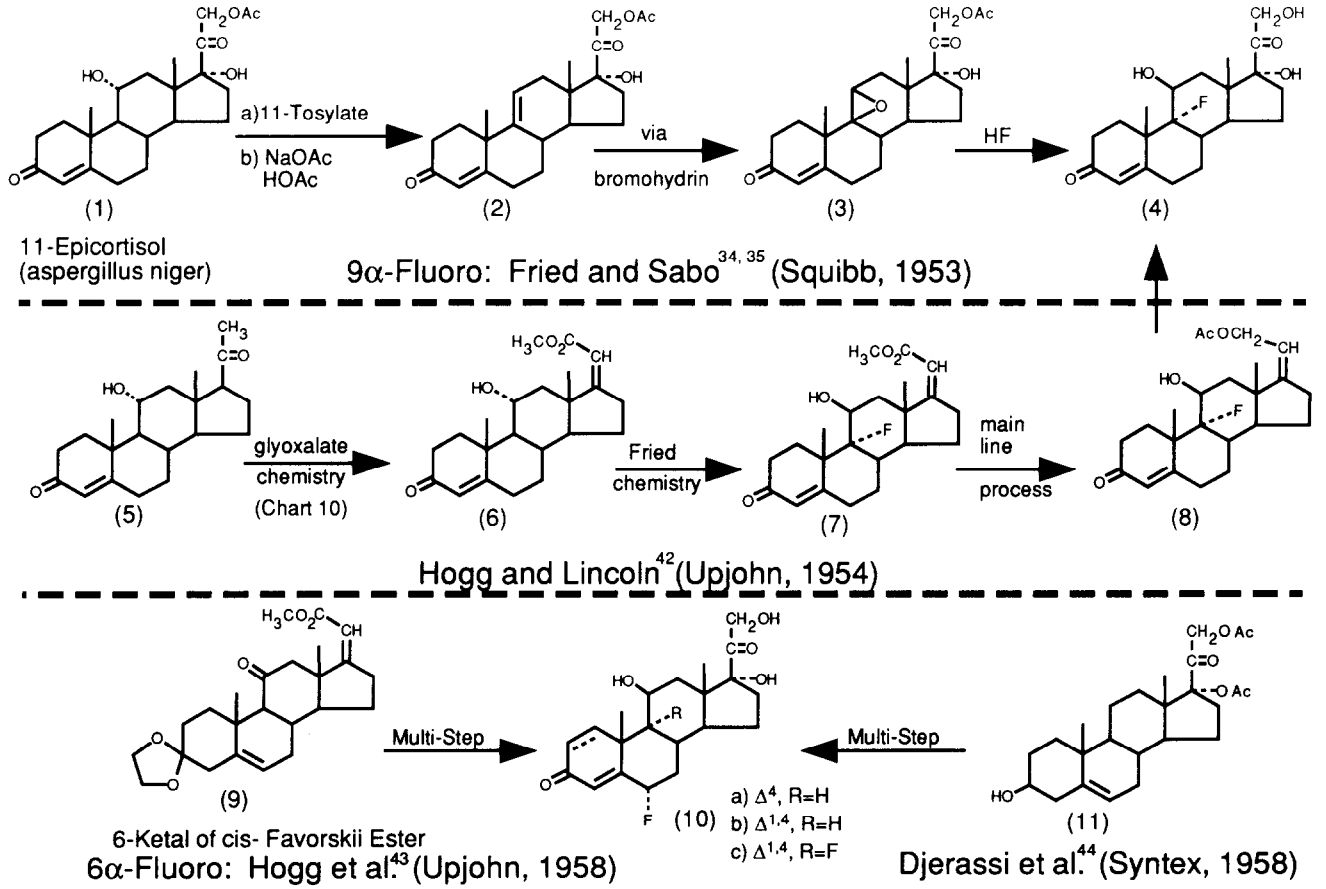


Chart 15 Halogen analogs: discovery and synthesis from Upjohn main-line intermediates.

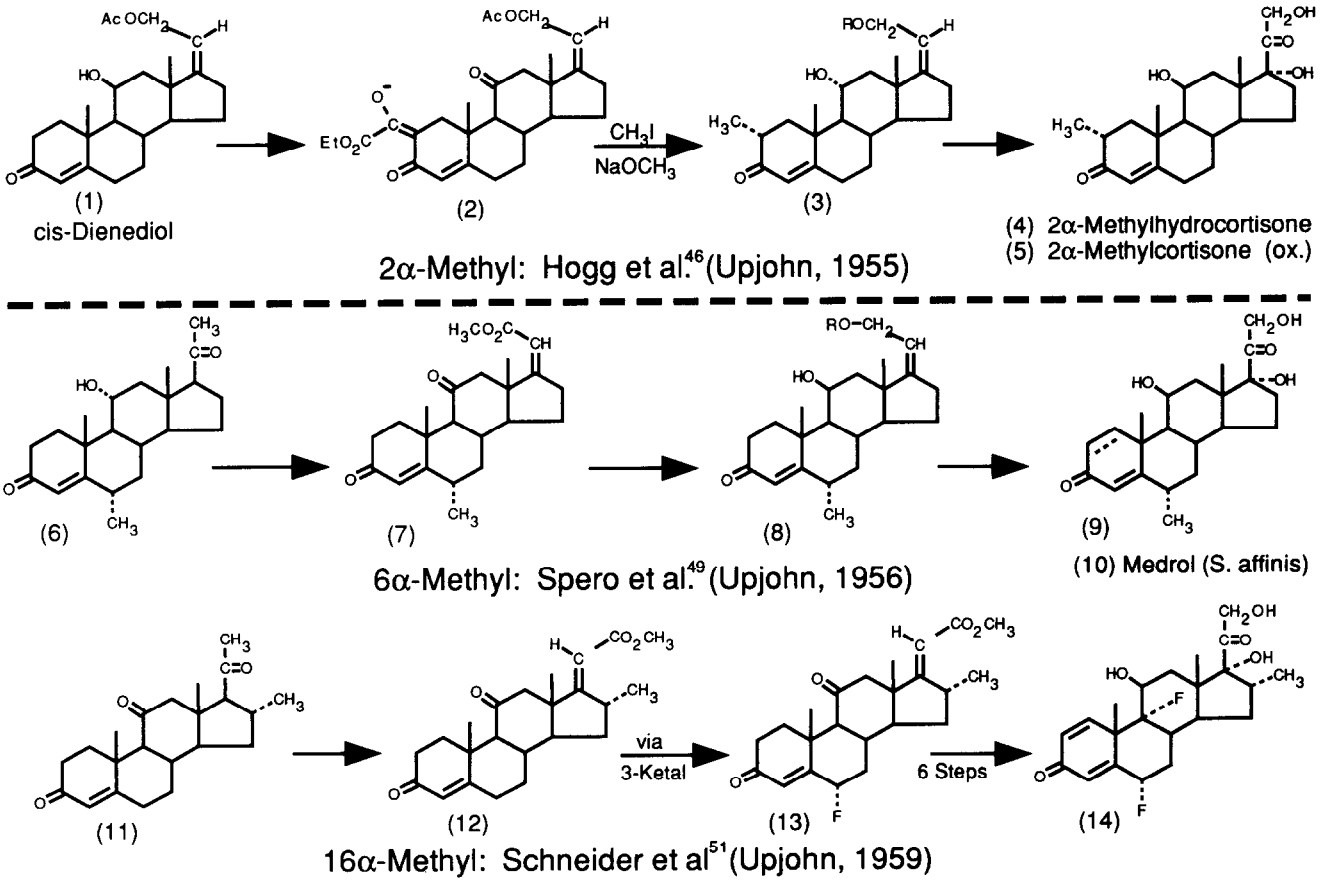


Chart 16 Alkyl analogs: discovery and synthesis from Upjohn main-line intermediates.

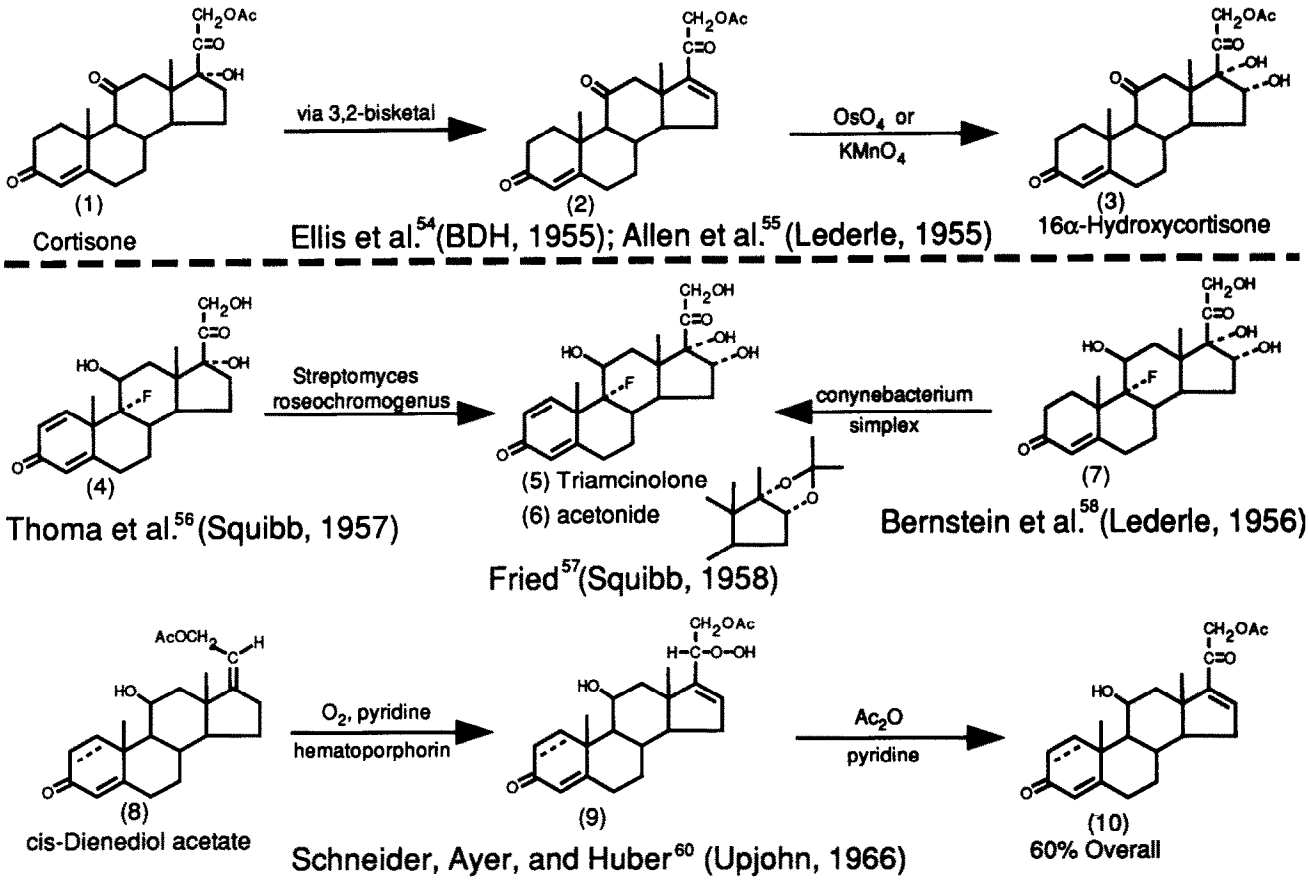


Chart 17 16-Hydroxyl analogs: discovery and synthesis from Upjohn main-line intermediates.

ery of these few important basic activity-modifying substituents; and (b) examples that demonstrate how each can be integrated into Upjohn's main-line process, thus illustrating process versatility.

The development and marketing of prednisolone,[36,37] 1-dehydro hydrocortisone (Chart 14, **6**), the first analog with improved therapeutic indices to reach the market (1955), was clearly a Schering first. But serendipity twice played a role in its prior history, once at Upjohn and once at Schering.

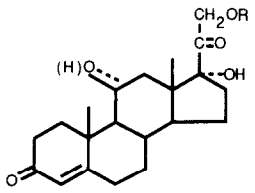
At Schering, as recorded by Sarett,[38] prednisolone was the result of an attempt to use the bacteria *Corynebacterium simplex* to hydrolyze a stubborn 11β-hydroxyl acetate. Instead, both hydrolysis and 1-dehydrogenation occurred, yielding prednisolone (**6**) directly and unpredictably. The importance of one-step microbiological techniques was again demonstrated.

At Upjohn, the 1-dehydro precursor (Chart 14, **4**)[33] to prednisolone was synthesized early in the proof of structure of a main-line process intermediate (as was also shown in Chart 11). Then the synthesis of prednisolone followed from the 1-dehydro Favorskii ester by the main-line process (Chart 14, **4–6**). Alternatively, 1-dehydrogenation of dienediol acetate by *Septomyxa affinis* (**2–5**) completed the tie-in with main-line intermediates. Murray, working with Peter D. Meister, found that it was necessary to use impure dienediol for this enzymatic 1-dehydrogenation with *S affinis*, suggesting the need for enzyme inducers, as described in examples 1–6 of reference 39. This set a pattern of mutual collaboration between the chemical and microbiological groups. The direct 1-dehydrogenation of hydrocortisone by *C simplex* remained the preferred commercial route, and patent rights went to Schering after a protracted interference.

It is noteworthy that this important analog substituent, coming so early and involving twin incidents of serendipity, was eventually to be incorporated into almost all of the important corticosteroid analogs that are on the market today (Chart 18).

The prior Fried and Sabo discovery (1953) of the potent 9α-fluorohydrocortisone was an event that had far reaching significance. Shown here in Chart 15 (**1–4**), it taught others how to incorporate 9 α-fluorine in combination with their own discoveries, if any, and basically reassured many that analog synthesis could be a fruitful field.

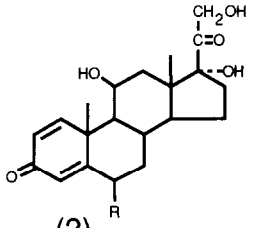
This earliest of the important enhancement discoveries was in itself not of therapeutic value, due to salt retention, yet it became a component in nearly all of the ultrapotent corticoids to be marketed by virtue of the attenuation of this unwanted side reaction by other



(1)

Hydrocortisone and derivatives

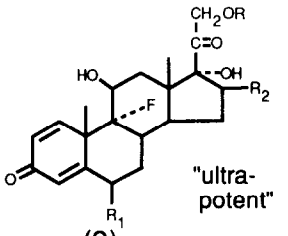
(11-Keto = Cortisone;
11-OH = Hydrocortisone)



(2)

Prednisolone and analogs

a) R = α-Methyl (Medrol, Upjohn)
b) R = α-Fluoro (Alphadrol, Upjohn)

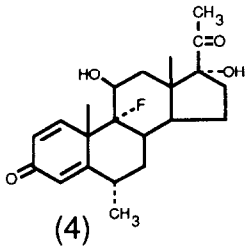


"ultra-potent"

(3)

9α-Fluoroprednisolone analogs

a) R_1 = H, R_2 = β-Me (Betamethasone, Schering)
b) R_1 = H, R_2 = α-Me (Dexamethasone, Merck & Schering)
c) R_1 = α-F, R_2 = β-Me (Diflorasone, Dermik)
d) R_1 = H, R_2 = OH [16,17 acetonide] (Triamcinolone, Lederle & Squibb)
e) R_1 = α-F, R_2 = OH (Fluocinolone, 16,17 acetonide, Syntex)



(4)

Oxylone (Upjohn)
Dulin et al. (1958)[62]

Chart 18 Major corticosteroid hormone products [incomplete, e.g., 9-chloro analogs such as beclomethasone (Glaxo) and dichlorasone (Schering)].

substituents. At Upjohn one school of thought saw this problem (and the same later with 2α-methyl hydrocortisone) as a negative factor in pursuing the potency race.

Serendipity had also played a role in the 9α-fluoro discovery. The Squibb group had independently discovered the 11α-hydroxylation of Reichstein substance-S by *Aspergillus niger*.[40] This publication appeared immediately after that of Upjohn on the 11α-hydroxylation of progesterone by *Rhizopus*, dispelling the temptation to be complacent. Attempts by the Squibb group to invert this 11α-hydroxyl to 11β-hydroxyl[41] led them eventually to 9α-fluorohydrocortisone by a combination of chance and rational design.

An example of the integration of the Fried chemistry into Upjohn processes is shown in the middle sequence of Chart 15 (**5** to **8** to **4**).[42]

In contrast, 6α-fluorination (**9** to **10** and **11** to **10**) enhanced corticoid activity significantly without attendant side effects. Although Upjohn and Syntex announced the synthesis of the 6α-fluorocorticoid family simultaneously[45] in 1958, U.S. patents on the disclosed 6-fluoro steroids (10a,b,c) were issued to Upjohn. Each company later developed and marketed a 6α-fluorinated product, Alphadrol[43] and Fluocinolone,[44] respectively, as listed in Chart 18. G. B. Spero and John Thompson synthesized 6α-fluorohydrocortisone (10a). 6α-Fluoroprednisolone (Alphadrol, 10b) was then produced microbiologically by *S affinis* (Murray).

2α-Methyl hydrocortisone (Chart 16), the first of the alkyl analogs, was synthesized by Lincoln in the Upjohn laboratories[46,47] shortly after the Fried and Sabo publication on 9α-fluorination. The glyoxalate strategy was used for its synthesis as shown (**1–4**). It was thought that steric effects by small nonpolar groups could be important in blocking inactivation by metabolic reduction of ring A, thus enhancing activity.

Indeed, activity was enhanced fivefold due in part to metabolic stabilization of ring A,[48] but so was mineralocorticoid activity. Curiously, the corresponding cortisone analog (**5**) was inactive. Presumably, metabolic reduction of the 11-ketone to the active 2α-methyl hydrocortisone (**4**) is also blocked, according to Upjohn's E. Myles Glenn and others. Such a metabolic interconversion of the 11-ketone to 11β-hydroxyl in vivo is known to be responsible for the activation of the otherwise inactive cortisone.

The synthesis of 6α-methyl hydrocortisone and its 1-dehydro derivative[49] was a logical extension of the 2α-methyl work. One synthesis by main-line procedures from 6α-methyl-11-keto progesterone is shown in Chart 16, **6** to **10**. 6α-Methyl prednisolone, or Medrol (**10**), more active than prednisolone with a better therapeutic index with respect to salt retention,[62] became Upjohn's most important corticoid product and remains so today, including its 21-succinate (Solu-Medrol). G. B. Spero spearheaded the synthesis of 6α-

methyl hydrocortisone, collaborating with Murray for its microbial 1-dehydrogenation to Medrol by *S affinis*. Essential to this oxidation was the use of 3-ketobisnor-4-cholenaldehyde (**5**, Chart 7) as an enzyme inducer, developed by Murray, which itself is 1-dehydrogenated and then reduced at C-22. Later the 1-dehydrogenation was integrated into the main-line chemistry at an earlier stage (i.e., 6α-methyldiendiol, **8**) in collaboration with O. Sebek.

The same strategy had been initiated for the synthesis of 16α-methyl hydrocortisone, starting with 16α-methyl-11-keto progesterone (**11**, Chart 16), when Merck and Schering came out at nearly the same time with the first 16 methyl analogs, e.g., dexamethasone (16α-methyl, 9α-fluoro prednisolone), listed in Chart 18. However, Upjohn's Schneider et al. then redirected their synthesis goal to include incorporation also of the 6α-fluoro group, yielding 6α,9α-difluoro-16α-methyl prednisolone (**14**), probably the most potent of any corticoid analog (×400) to date. Again Upjohn[51] and Syntex[52,53] announced this same potent analog in the same issue of the same journal.

British Drug Houses[54] and Lederle[55] synthesized 16α-hydroxycortisone (Chart 17, **1** to **3**), 16α-hydroxy was the remaining of the key activity modifying substituents to be discovered. Lederle reported that antiinflammatory potency was lowered but salt retention was eliminated. This attenuation characteristic carried over advantageously in combination with other potent enhancers.

Squibb[56,57] and Lederle[58] at about the same time converged on the same target, triamcinolone (**5**), each by a different microbiological last step, 16α-hydroxylation or 1-dehydrogenation. It should be noted that Fried and co-workers[59] had earlier pioneered the microbiological 16α-hydroxylation of progesterone, published just 1 month after Upjohn's 11α-hydroxylation of progesterone.[3–5] It is, of course, only academic to consider the outcome had the luck of the draw been reversed.

Upjohn was not involved in the 16α-hydroxy analog development; that is, not until W. P. Schneider et al. devised an important process for their synthesis.[60] Dienediol acetate, Upjohn's main-line intermediate, was converted to the 16-dehydro precursor of 16α-hydroxy corticoids (**10**, Chart 17) in 60% overall yield by photooxidation in pyridine and treatment of the intermediate peroxide with acetic anhydride. This enabled Upjohn to become a major producer of the 16α-hydroxy steroids and, as we will see later, of the 16α-methyl steroids as well.

The principal anti-inflammatory steroid analogs to reach market status are summarized in Chart 18 along with the natural hormones, not including important derivatives (prohormones) such as Upjohn's hydrocortisone 21-succinate (Solu-Cortef) or Solu-Medrol.

They are grouped as shown to highlight the facts that (a) the 1-dehydro substituent is common to all of the analogs (groups 2, 3, and 4), and that (b) the "ultrapotent" groups (3 and 4) all contain the 9α-fluoro substituent in addition to 1-dehydro. The importance of these two substituents is matched by the development of the methyl and 16-hydroxy substituents. Most if not all of these steroid analogs are manufactured today for the industry by Upjohn.

Oxylone (**4**),[61] shown separately in Chart 18, became Upjohn's sole product in the ultra-potent category as a topical antiinflammatory agent. Upjohn's W. E. Dulin et al.[62] demonstrated that in this steroid (6α-methyl-9α-fluoro-21-deoxyprednisolone or Oxylone) removal of the 21-hydroxyl group had reduced glycogen activity to approximately one fifth but increased antiinflammatory potency approximately fivefold. This foretold a reduction of unwanted systemic glucocorticoid activity when used topically as an anti-inflammatory agent.

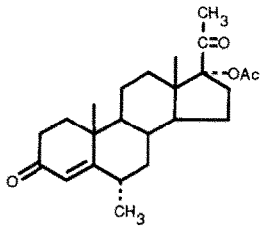
An extensive endocrine evaluation program, largely conducted by Dulin and E. Myles Glenn et al., matched the chemical effort and helped to guide the direction of analog synthesis at Upjohn. The endocrine evaluation program was headed by endocrinologists R. O. Stafford, W. W. Byrnes, and J. C. Stucki, each in his turn throughout the 1950s. All three trained at the University of Wisconsin under R. K. Meyer, a consultant and, interestingly, one of Heyl's early postdoctorates.

Upjohn in the mid-1950s was unprepared to offer products for contraception, so that the first orally active progestin, 17α-acetoxyprogesterone, was instead developed for pet use in the veterinary division. However, two further progesterone analogs both containing the 6-methyl group (Chart 19, **1** and **2**) were later marketed by Upjohn. One of these, melengesterol acetate (**2**),[63] a potent progestin, first synthesized by J. Alan Campbell, was introduced in the veterinary field, but as a growth promoter in cattle. The other, medroxyprogesterone acetate (Provera)[64] despite its acceptance as a contraceptive for use in humans in 90 countries, failed to win FDA acceptance until very recently, a victim of political more than medical considerations. Medroxyprogesterone acetate was synthesized in the mid-1950s by J. C. Babcock, chief chemical advocate in the search for orally active progesterone analogs for contraception. Stucki, chief biologist for the contraception program, picked up the baton around 1957 to promote support for the medical, regulatory, and marketing phases, still ongoing.

History repeats itself

By the late 1960s the discarded sitosterol stockpile had become enormous, as was noted earlier (Figure 10). J. Ward Greiner (Figure 11) was still promoting sitosterol utilization, including such gimmicks as distributing dollar bills in jars labeled sitosterol as "seed" money reminders, and mustering support to oppose pressures to get rid of the pile in order to free up the space. Greiner was supported by Schreiber; and encouraged by Weisblat who became more than any other single person responsible for the rich research environment that evolved at Upjohn.

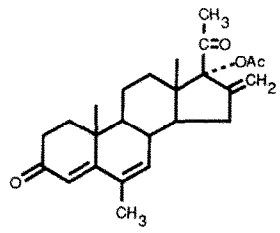
The sitosterol utilization project, based on chemical or microbiological methods, or utilization per se, was formally initiated in the early 1970s. The outcome



(1)
Medroxyprogesterone acetate

oral (Provera) and parenteral (Depo-Provera) contraceptives

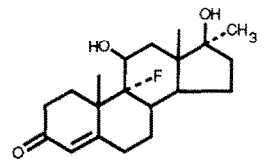
Babcock et al. (1958)[64]



(2)
Melengesterol acetate

veterinary product for estrus synchronization and weight gain in cattle

Babcock and Campbell (1967)[63]



(3)
Fluoxymesterone (Halotestin)

anabolic agent; androgenic hormone therapy

Herr et al. (1956)[65]

Chart 19 Upjohn progestational and androgenic products.

(Chart 20) was just as dramatic as the events that had brought Upjohn to this point in the first place. The sitosterol pile was resurrected, as we will see, by the discovery and development of yet another combination of microbiological/chemistry processes, this time based on sitosterol, supplementing those that had been in use for a quarter century based on progesterone from stigmasterol. The radical bacterial degradation of sitosterol to 9α-hydroxyandrostenedione (Chart 20, **2–5** to **7**) by Upjohn's Merle Wovcha et al.[67] was the key to this sitosterol utilization breakthrough.

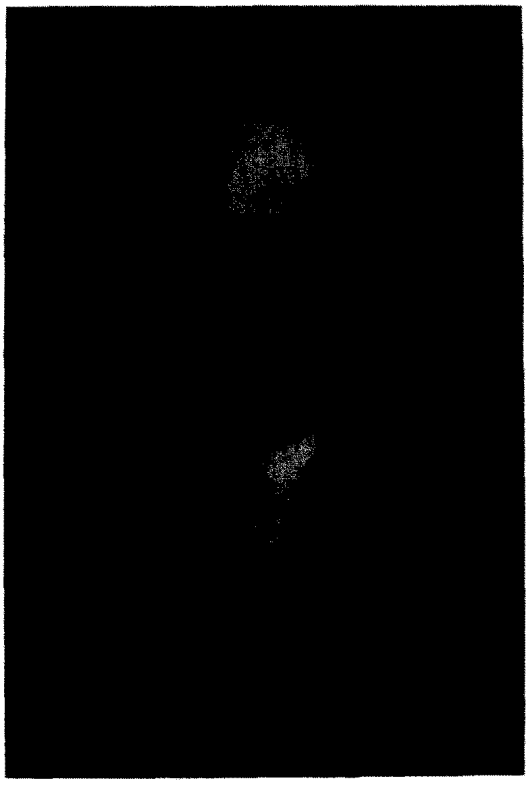


Figure 11 J. Ward Greiner.

Wovcha discovered a mutant of *Mycobacterium fortuitum*, a potent sterol degrader, which lacked the enzymes to go beyond 9α-hydroxyandrostendione in sitosterol degradation. In a single microbiological step, both sterol side-chain degradation and 9α-hydroxylation of ring C were accomplished, the latter usable for the required 11-oxygenation. In contrast to the 11α-hydroxylation of progesterone, the goal for this microbial degradation of sitosterol (9 OH-AD) was postulated and targeted specifically, based on knowledge gained from the elaboration by the steroid community of pathways in the microbial assimilation of sterols. Marsheck et al.[66] at Searle had earlier found a mutant of *M fortuitum* for the microbial production of androstenedione (**5**), another intermediate in the pathway.

But the latter intermediate (AD) lacked the important foothold needed for introducing oxygen at position-11, which 9α-hydroxy AD provided. The tricky dehydration of 9α-hydroxyandrostenedione to $\Delta^{9(11)}$-androstenedione (**9**) (exclusively) by Shephard,[68] using Cl SO_3H assured the fulfillment of that requirement.

The exercise of sound strategy and a bit of help from the luck of the draw enabled Upjohn to be favored by a second important microbiological breakthrough. Given well-integrated chemical processes to exploit this remarkable degradation, the extensive steroid microbial methodology that had accummulated in the community at large still offered no better solutions.

New chemistry based on $\Delta^{9(11)}$-androstenedione was devised in Chemical Process Research for the regenera-

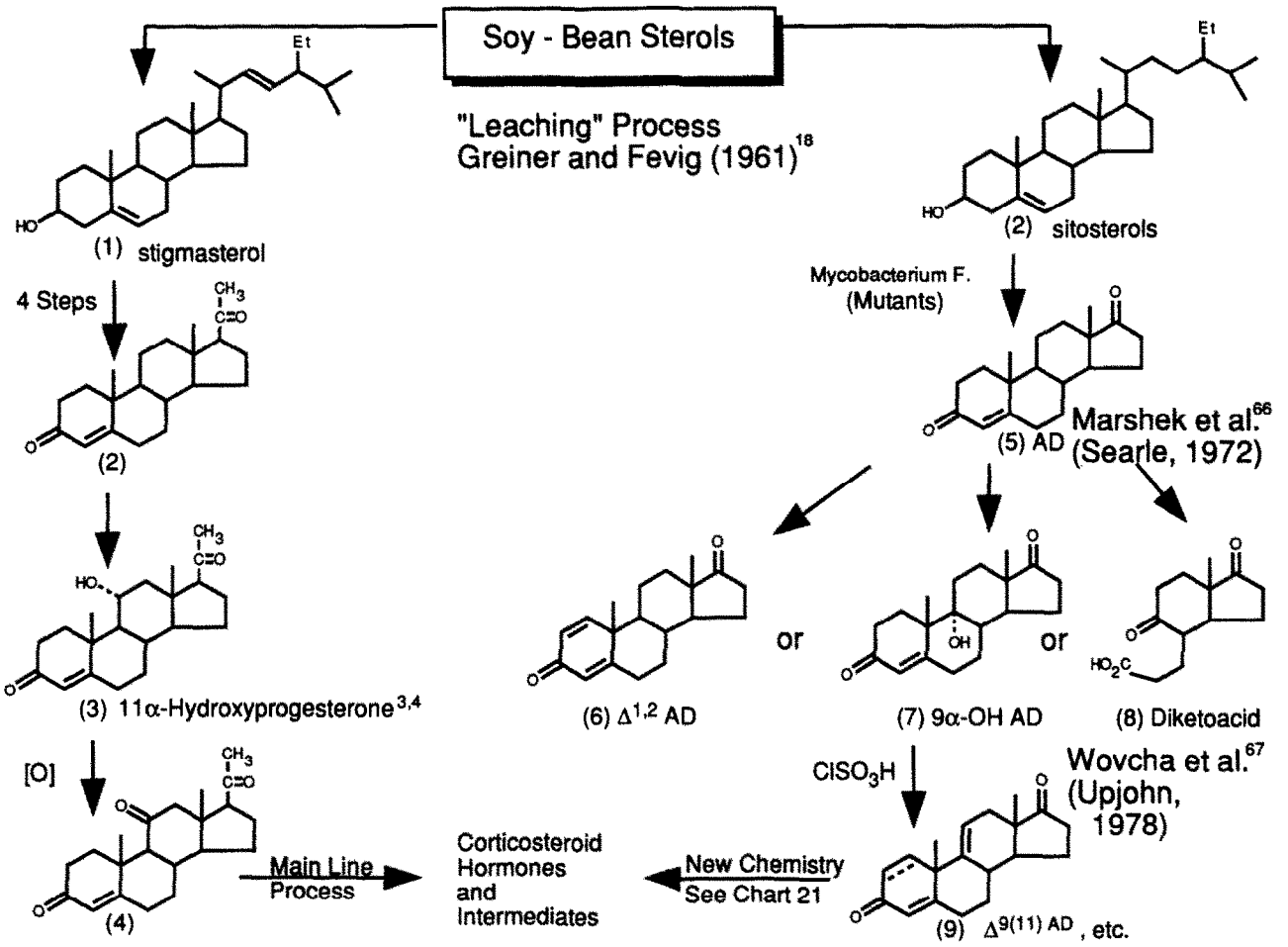


Chart 20 Sitosterols resurrected: history repeats itself.

tion of the pregnane side chain and further synthesis of the Upjohn family of corticosteroids as shown in Chart 21 (reviewed by Livingston[69]).

Not one, but three 17-side chain regenerating schemes were developed. Two are shown: the chloroaldehyde process of Hessler and Van Rheenan[71] and the silicon nucleophile annelation process (SNAP) described by Livingston.[70] The third, the allene sulfoxide process of Van Rheenan and Shephard,[72] is not shown.

Note that the chloraldehyde process (on the right) generates the same 16-dehydro intermediate (**5**) that had become available earlier from the main-line process, as previously seen in Chart 17. Therefore, the three separate procedures shown here for completing the full cortical side chain, with or without 16α-hydroxyl or 16α-methyl substituents, had already been developed by the authors.

The SNAP process on the left side of Chart 21, another truly elegant procedure, was adapted to the regeneration of all three of the basic pregnane side chains, **11, 12,** and **7**. Only the latter, the full corticoid side chain (**7**), is produced in common by both side-chain regeneration procedures.

In overview, two complementary sets of combined microbiological/chemical processes, each based on one of the two sister sterols, stigmasterol and sitosterol, both derived from a single and abundant plant source, the soybean, have evolved over 40 years at Upjohn (Chart 20). 11α-Hydroxyprogesterone (**3**) on the left and 9α-hydroxyandrostenedione (**7**) on the right are the microbiologically produced counterparts in their respective pathways. $\Delta^{9(11)}$-Androstenedione (**9**) now shares the limelight with 11 keto-progesterone (**4**) in well-integrated processes as an intermediate for the synthesis of the full family of steroid hormones, analogs, and intermediates that now flow from the Upjohn production "tree."

Given that members of the genus *Rhizopus* of the family Mucoraceae are ubiquitous and 11α-hydroxylate those steroids which are also the logical choices as substrates, it seems clear that this discovery would have been made sooner or later in another prepared environment had it not been at Upjohn. To such comments as, "It is incredible that it happened so quickly," Murray replies, with characteristic humility, "You'd better believe it."

Nevertheless, the 11α-hydroxylation of progesterone was the trigger shortly after the beginning of the

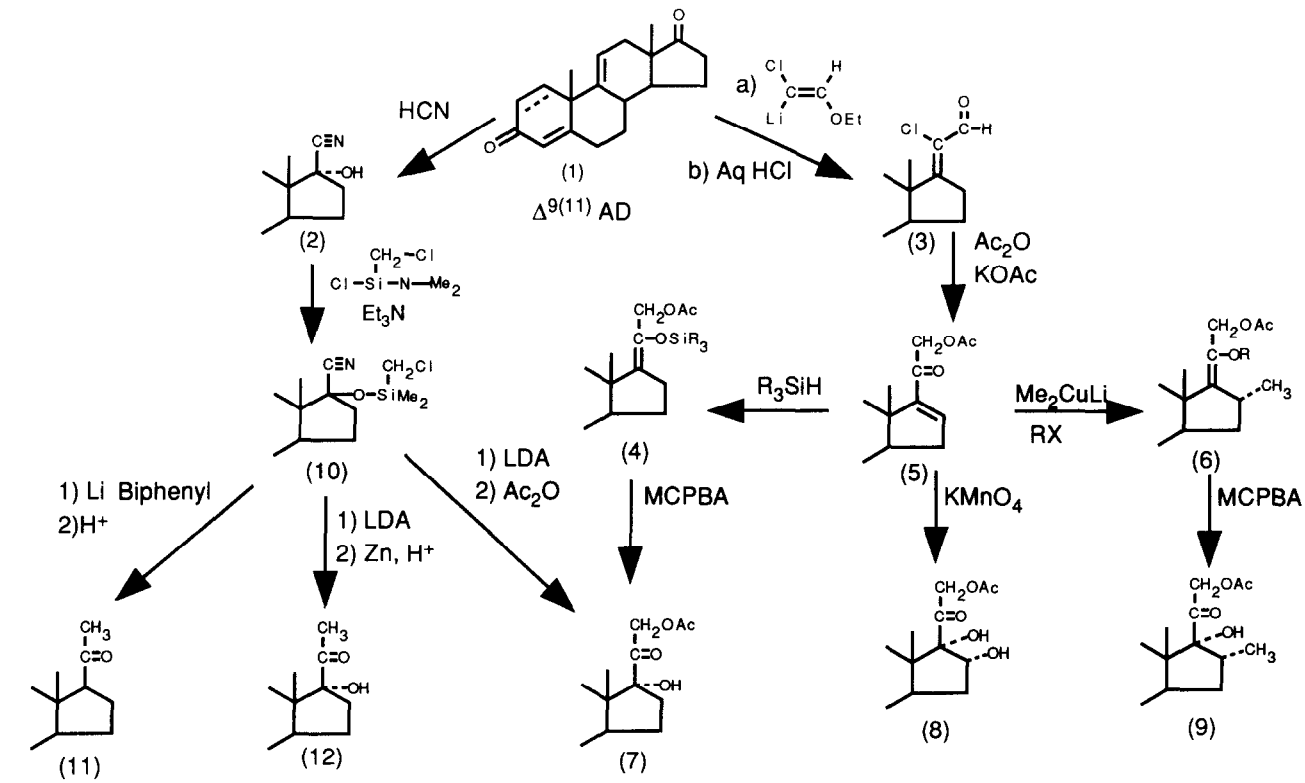


Chart 21 New and novel D-ring chemistry.

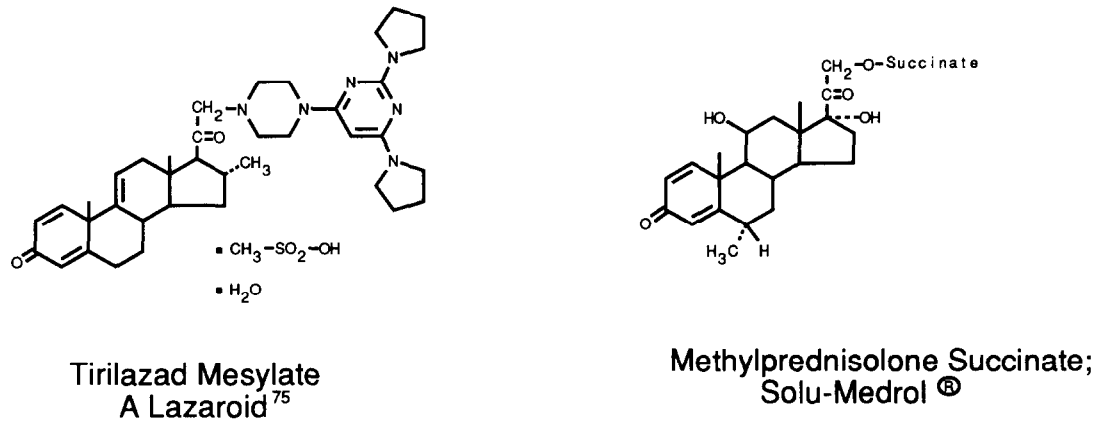


Chart 22

cortisone era in 1949 that reshaped steroid research strategy throughout the pharmaceutical industry. Strategy at Upjohn was redefined over time by a succession of equally determinant innovations. In macroview it can be seen that the overall anatomy of the company's steroid technology today is a composite of 40 years of such innovation, thus fulfilling the promise of an underlying strategy of integration.

History in the making

Steroid history is still in the making at Upjohn, where a "new pharmacology" has evolved,[73,74] hinging on the ability of glucocorticoids to inhibit lipid peroxidation by mechanisms that are quite apart from classical glucocorticoid mechanisms, and promising new treatment for central nervous system trauma and ischemia.

Solu-Medrol (Chart 22), widely used intravenously to treat or forestall adrenocortical insufficiency, was shown to inhibit peroxidation in spinal cord sections (in vitro) as well as in vivo at high doses in injured spinal cord sections (in cats). This was confirmed later in experimental studies in humans.

To eliminate the classical glucocorticoid properties, a synthetic program based on the steroid intermediates readily available at Upjohn led to a new class of 21-amino steroids[75] with the desired activity split. The structure of tirilazad mesylate, the lead compound of the so-called lazaroids, is shown in Chart 22.

The lazaroid development adds a new dimension to the anatomy of Upjohn steroid technology sculptured over a 40-year period and clearly demonstrates the enabling power of the axiom "Build on Strength."

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