Endocrine Activity of Plant-Derived Compounds: An Evolutionary Perspective

(43845)

MICHAEL E. BAKER[1]

Department of Medicine, University of California, San Diego, La Jolla, California 92093-0623

Abstract. **Although plants have long been known to have important pharmacological effects in humans, the mechanism by which plant-derived compounds act in humans is still being elucidated. Two important pathways for the biological actions of plant-derived compounds involve binding either to hormone receptors or to enzymes that metabolize hormones. What are the origins of this interaction between plant-derived compounds and animals? And what insights can we gain from investigating this question? Some answers come from recent sequence analyses, revealing that 17β-hydroxysteroid dehydrogenase, which regulates estrogen and androgen levels in humans, and 15-hydroxyprostaglandin dehydrogenase, which regulates prostaglandin E_2 and $F_{2\alpha}$ levels in humans, have a common ancestor with proteins in *rhizobia* that are important in forming nitrogen-fixing nodules in legume roots, and 3β-hydroxysteroid dehydrogenase, which regulates progestin and androgen levels in humans, has a common ancestor with enzymes important in the synthesis of anthocyanins. This evolutionary kinship, when combined with the structural similarities between flavonoids, licorice-derived compounds, and steroid hormones, provides another perspective on the hormone-like activity of flavonoids and other plant-derived compounds in humans: some of the hormone-like activity of plant-derived compounds is due to binding to steroid and prostaglandin dehydrogenases.**

[P.S.E.B.M. 1995, Vol 208]

Plants have been used as medicines for thousands of years (1–5). In ancient times, the Assyrians, Babylonians, and Egyptians used various parts of plants for treating diseases and ailments. The writings of Hippocrates, Theophrastus, Pliney the Elder, and Galen, and texts from China and India discuss the importance of plants for treating various ailments. This information was considered such an important cultural resource that it was carefully transmitted from generation to generation. A major change in this attitude occurred in 20th Century, when antibiotics and other drugs were discovered and used for treating infectious diseases, and the medicinal uses of plants fell into disfavor in industrialized countries. The collective wisdom of thousands of years of folk medicine was put into a category of superstitious information, with marginal value, and little scientific validity.

However, as we prepare to enter the 21st Century, a change in the status of folk medicine is occurring as pharmaceutical companies, university scientists, and agricultural agencies are rediscovering the value of plants in medicine. An important impetus for this change came from the discovery in Australia that flavonoids in plants have estrogenic activity in foraging animals such as sheep (6, 7). Later studies showed that plant-derived compounds have hormone-like activity in rats (8–10), rabbits (11), tumor cells (12–14) and even fish (15). These reports stimulated epidemiological studies to analyze the diet of humans which revealed that the presence or absence of certain plants in the diet influences the incidence of diseases such as cancer (16–22), which often require estrogens or androgens for growth. Other plant-derived compounds bind to enzymes that are important in regulating ste-

[1] To whom requests for reprints should be addressed at Department of Medicine, 0623B, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0623.

0037-9727/95/2081–0131 $10.50/0

This article is dedicated to Jim Duke, pioneer and gadfly in developing our understanding of the importance of plants in human physiology.

roid hormone (23–29) and prostaglandin (29–34) action in humans. The evidence for hormone-like activity of plant-derived compounds in these reports from laboratories throughout the world verifies many of the medicinal uses of plants reported by Greek and Roman writers, giving these early writings "scientific respectability."

In this paper, I use an evolutionary approach to understand the endocrine actions of plants. This evolutionary perspective is a thread that connects the action of flavonoids synthesized by soybeans to promote the formation of nitrogen-fixing nodules by soil bacteria (35–39), the synthesis of anthocyanins in flowers (40, 41), the extract of the licorice root, and the mechanisms that regulate steroid hormone and prostaglandin action in humans (29, 33, 42, 43). With this approach, I seek to provide another perspective on plants' hormone-like actions in humans that can be useful in preventing and treating diseases in humans.

Cell-Cell Communication in Humans

Hormone Receptors. Vertebrates, from fish to humans, use hormones as messengers to regulate physiological processes in target organs. Hormones act by binding to receptor proteins in the target cell, which leads to transcriptional regulation of different gene products and the desired physiological response. Hormones regulate diverse physiological processes such as development, reproduction, metabolism, and homeostasis. Steroids and prostaglandins, the two classes of hormones that will be discussed here, have important roles in these processes in humans.

Dehydrogenases Regulate Steroid Hormone and Prostaglandin Action. It is only in the last few years that the role of enzymes that metabolize steroids and prostaglandins in regulating the actions of these hormones has been appreciated. For example, 11β-hydroxysteroid dehydrogenase regulates glucocorticoid action by catalyzing the interconversion of hydrocortisone and cortisone, an inactive steroid (Fig. 1). In the liver, an important site for glucocorticoid synthesis, 11β-hydroxysteroid dehydrogenase acts as a reductase to reduce the C11 ketone on cortisone.

In the kidney the reverse reaction predominates: hydrocortisone is oxidized to cortisone, a reaction that prevents circulating hydrocortisone from occupying kidney mineralocorticoid receptors and regulating transcription of mineralocorticoid responsive genes. Aldosterone is inert to 11β-hydroxysteroid dehydrogenase and can regulate mineralocorticoid responsive genes in the kidney.

Similar considerations hold for 17β-hydroxysteroid dehydrogenase, which catalyzes the interconversion of estradiol, the biologically active estrogen, and estrone, a much weaker estrogen. This enzyme also catalyzes the interconversion of testosterone and

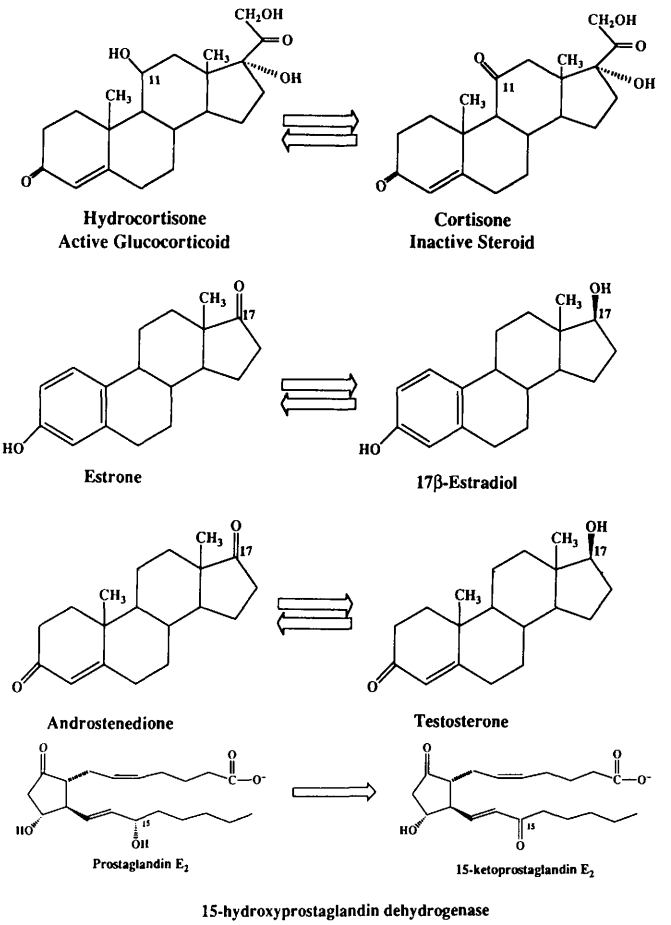


Figure 1. Metabolism of steroids and prostaglandins by sec-alcohol dehydrogenases. 11β-hydroxysteroid dehydrogenase catalyzes the interconversion of the active glucocorticoid hydrocortisone and inactive cortisone. 17β-hydroxysteroid dehydrogenase catalyzes the interconversion of estradiol and estrone. Estrone is a weaker estrogen than estradiol. 15-hydroxyprostaglandin dehydrogenase inactivates prostaglandin E_2 by catalyzing the oxidation of the C15 alcohol to a ketone.

androstenedione (Fig. 1). Testosterone is a precursor of dihydrotestosterone, the active male reproductive hormone, and of estrogen. Thus, plant-derived compounds that inhibit 17β-hydroxysteroid dehydrogenase will have important effects on female and male reproductive function and development. Another enzyme relevant to this discussion is 15-hydroxyprostaglandin dehydrogenase, which catalyzes the oxidation of C15 alcohol on prostaglandin E_2 and $F_{2\alpha}$, which is the mechanism of inactivation of these hormones (44–46). Compounds that inhibit this enzyme will have prostaglandin-like activity.

From the above we can see that expression of enzymes that promote either the synthesis or degradation of hormones, such as estrogens, androgens, and prostaglandins, is one of the mechanisms for regulating these signals' physiological actions. An important consequence of this mechanism for regulating hormone action is that compounds that inhibit these enzymes appear to be acting as a hormone or an antihor-

mone. This effect can mistakenly be interpreted as meaning that the compound acts by binding to a hormone receptor. The reality is that they are binding to hormone-metabolizing enzymes.

Cell-Cell Communication in the Rhizosphere

Chemical Warfare in the Rhizosphere. The rhizosphere, the underground, that unseen world usually ignored by atmosphere chauvinists, is teaming with life: bacteria, fungi, invertebrates, and vertebrates live there and interact with each other in a variety of ways. As with life above ground, life's major activities are consuming other organisms, defending against attacks, and claiming territory for growth and reproduction. Polyketides, a class of secondary metabolites, are an important weapon in the arsenal for underground warfare (47–49). For example, Streptomyces and other Actinomyces secrete polyketide antibiotics into the soil that kill other bacteria. Humans use these polyketides for controlling bacteria, and this is likely to be one role in the soil, although other functions have been suggested, for antibiotics (29, 50–53).

Peaceful Coexistence in the Rhizosphere. Plants also secrete compounds into the soil to protect against insects or bacteria, or fungi, especially after wounding (54). One class of secreted compounds,the flavonoids, is of special interest (40, 41) because they are also used for peaceful activities, as signals to initiate a cooperative activity: symbiosis between soil bacteria belonging to the Rhizobium family and legumes (35–39) that leads to formation of nitrogen-fixing nodules in the legume root. These nodules fix atmospheric nitrogen into ammonia, which is then converted into glutamine for use by the plant. As such, the symbiosis provides an important selective advantage for soybeans, peas, beans, and other legumes: they can make their own nitrogen fertilizer from the atmosphere and grow in nitrogen-deficient soil.

Due to the economic importance of this process, much research has been devoted to characterizing and understanding the process of signaling between legumes and rhizobia that leads to nodule formation in roots. In fact, this is one of the best understood interactions in the rhizosphere (35–39).

Flavonoids and Nod Factors: Signals for Legume-Rhizobia Communication. The signals for communication between legumes and rhizobia are flavonoids and nod factors (Fig. 2). A flavonoid, secreted by the host plant, acts as a chemoattractant to direct the appropriate rhizobium to the plant root. The flavonoid binds to a specific protein (called NodD) in the host rhizobium. This binding increases the affinity of this protein for DNA leading to transcription of a group of nodulation (nod) genes. These gene products are used by the bacterium to synthesize a molecule, called a nod factor, that acts on the plant root to facilitate the formation of the nitrogen fixing nodule. Thus, the plant synthesizes a flavonoid that induces gene transcription in a bacterium, that leads to the bacterium synthesizing a signal that acts on the plant to promote nodule formation.

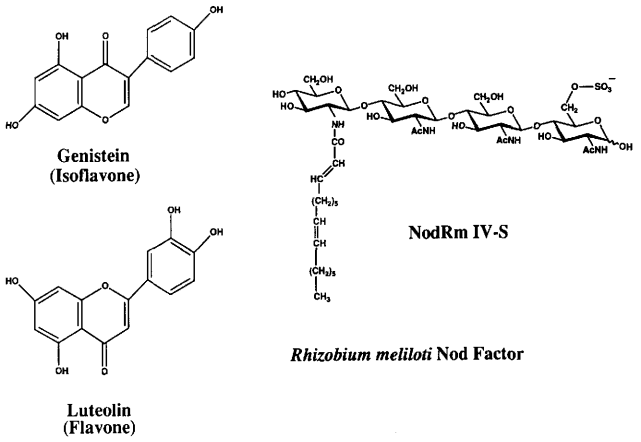


Figure 2. Structure of flavonoids and a nod factor.

The differences in the NodD proteins in different rhizobia provide specificity for the flavonoids. Interestingly, some flavonoids inhibit nodulation by rhizobia, acting as antinodulation signals. This "underground chemical warfare" prevents rhizobia from nodulating competing plants.

Similarities to Steroid Hormone Action in Humans. The legume-rhizobium signaling process has many properties that resemble steroid hormone action in humans. Steroid hormones bind to receptor proteins in target cells, which increases the receptor's affinity for DNA and leads to the regulation of gene transcription in the target cell. Steroids bind to specific receptor proteins to exert their biological effects; estrogens do not bind to progesterone receptors and vice versa. However, in some instances a receptor will bind more than one class of steroids. For example, progesterone and hydrocortisone have good affinity for mineralocorticoid receptor. Indeed, hydrocortisone occupancy of the mineralocorticoid leads to a strong response, similar to that found for the natural mineralocorticoid, aldosterone. On the other hand, progesterone occupancy of mineralocorticoid receptor leads to an inactive receptor. In this case, progesterone is acting as an antihormone, which is similar to the antinodulating activity of some flavonoids. Antagonistic steroids are of great interest for regulating endocrine-related diseases. For example, tamoxifen, a compound that binds to estrogen receptor, but does not have estrogenic activity has much promise for regulating estrogen-dependent tumors.

Structural Similarities Between Flavonoids and Estrogens and Androgens. The discoverers of the estrogenic activity of flavonoids in sheep noticed that flavonoids had some structural similarity to estrogens

(Fig. 1 and 2). Indeed, the similarities are striking and consistent with findings that some estrogen receptors (11–13) and type II binding sites (8), which recognize estrogens, bind flavonoids with high affinity. The type II binding site may be an eosinophil peroxidase (55), which case, the flavonoid's physiological activity is due to binding to an enzyme that is stimulated by 17β-estradiol. This notion, that steroids may bind with high affinity to enzymes, has received important experimental support from the recent cloning and sequencing of a *Candida albicans* protein that binds glucocorticoids with n*M* affinity, which indicates that this protein is an enzyme (56). This is of interest because, as described in the next section, plants and soil bacteria that are important in plant ecology contain enzymes that have sequence similarities to steroid and prostaglandin dehydrogenases. Together, this suggests that some hormone-like effects in humans of plant-derived compounds are due to their binding to enzymes that metabolize steroids and prostaglandins (29, 33, 42, 43).

Evolutionary Connections Between Rhizobia Proteins and Steroid and Prostaglandin Dehydrogenases

Amino Acid Sequence Similarities. Several years ago, we made the surprising discovery that the amino acid sequence of *R. meliloti* NodG was similar to human 17β-hydroxysteroid dehydrogenase (29, 57), the enzyme that metabolizes estrogens and androgens in humans (Fig. 1). Analysis of the sequences of these two proteins indicated that the probability of this similarity occurring by chance was less than 10^{-24}. This indicated that the proteins are homologs; that is, they are derived from a common ancestor. Later we found that *Bradyrhizobium japonicum* FixR protein is also homologous to 17β-hydroxysteroid dehydrogenase (50). All of these proteins are homologous to the animal 11β-hydroxysteroid dehydrogenase, 20β-hydroxysteroid dehydrogenase and 15-hydroxyprostaglandin dehydrogenase (58–61), as well as to a plant reductase which catalyzes a key step in chlorophyll synthesis (58) and a dehydrogenase which may regulate the action of a plant hormone (62). Furthermore, these proteins have a common ancestor with β-ketoreductases which are part of the enzyme complex for synthesizing polyketide antibiotics in soil bacteria (29, 33, 47, 50). Thus, this family goes back at least 2 billion years to the time when eukaryotes and prokaryotes diverged from a common ancestor.

Steroids Bind to Proteins in Yeast and Bacteria. In some instances, an animal hormone binds to a bacterial or fungal enzyme homolog despite the phylogenetic distance between these organisms. For example, *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase, a bacterial homolog of human 11β-hydroxysteroid dehydrogenase, metabolizes the C20 group on progesterone and cortisone, evidence that an animal hormone can bind specifically to a bacterial dehydrogenase that has some sequence similarity to animal dehydrogenases. Recently, Adamski's laboratory has sequenced a 17β-estradiol dehydrogenase (63) that has very strong sequence similarity to the trifunctional enzyme in *Candida tropicalis* (64). The similarity is strong enough to suggest that the fungal enzyme could recognize estradiol or another steroid and, in fact, could be one of the steroid binding proteins found in fungi (56, 65–68). This could explain the action of steroids in fungi, which is of medical interest due to the increasing prevalence of fungal infections.

The evidence that animal hormones bind to bacteria and fungal enzymes raises the following question: is the converse true for compounds that bind bacterial and plant dehydrogenases that have sequence similarity to animal dehydrogenases? That is, do hormone dehydrogenases in humans have a physiologically significant affinity for compounds synthesized by plants and bacteria? As mentioned above, flavonoids have similarities in structure to some steroids. Do flavonoids also interact with steroid dehydrogenases? In the next section, we discuss plant derived compounds with steroid-like structures that are found in the extract from the root of the legume *Glycyrrhiza glabra*. These compounds have important physiological effects in humans due to interaction with steroid and prostaglandin dehydrogenases.

Plant-Derived Compounds Have Hormone-like Activity in Humans

Licorice and King Tut. Licorice is an extract of the roots of *Glycyrrhiza glabra,* a legume that is widely distributed throughout the Mediterranean and Orient. Over 2000 years ago, licorice was used as an herbal for quenching thirst and promoting healing of ulcers (1–4). Licorice root was considered to be so significant that it was placed in King Tutankamen's tomb. As valuable as licorice was for the ancients, in the 20th century licorice's principal use has been as a flavoring agent; for the most part, its medicinal properties have been ignored.

Licorice and Addison's Disease. It was only recently that the compounds in licorice that are important in some of its healing activities were isolated. The compound with steroid like activity is glycyrrhizic acid (Fig. 3), which is metabolize by intestinal bacteria to its aglycone glycyrrhetinic acid, the biologically active species. Comparison of glycyrrhizic and glycyrhetinic acid with the steroid structures in Figure 1 reveals that these triterpenoids have some structural resemblance to steroids. We owe our present interest and understanding of licorice's medicinal properties to the pioneering efforts of Reevers (68) and Dr. S. Gottfried at

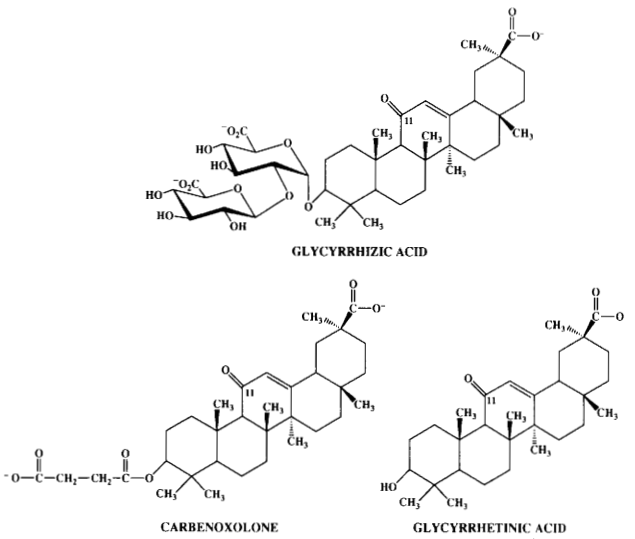


Figure 3. Structure of licorice-derived compounds.

Biorex (69), who were interested in herbal medicine. Reevers found that an herbal extract that contained licorice helped people with Addison's disease, which is caused by a glucocorticoid deficiency. Later studies showed that licorice acts by inhibiting 11β-hydroxysteroid dehydrogenase, which increases glucocorticoid levels (23).

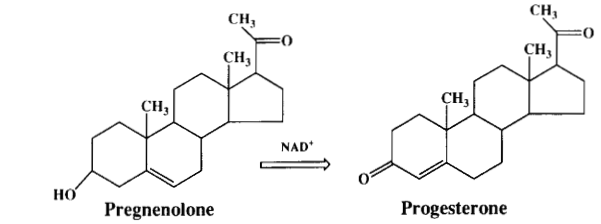
Licorice and Healing of Ulcers. Dr. S. Gottfried's interest in herbal medicine led him to read about licorice's use for treating ulcers and wounds in a botanical book, *Historia Botanica Practica,* published in 1774 (69). As a result, Dr. Gottfried initiated a program at Biorex that led to isolation of glycyrrhetinic acid, which has anti-inflammatory activity, mineralocorticoid-like activity and antiulcer activity (70–74). Because glycyrrhetinic acid is not very soluble in water, Dr. Gottfried's team at Biorex collaborated with Professor E. E. Turner at Bedford College, University of London, to synthesize soluble analogs of glycyrrhetinic acid with substituents at C3 (71). One of these analogs, carbenoxolone, (Fig. 3) is used as an antiulcer agent in Great Britain, under its commercial name of Biogastrone (71–73). We now know that licorice inhibits its 15-hydroxyprostaglandin dehydrogenase, which increases prostaglandin levels, promoting the healing of ulcers (34, 71–73).

Mineralocorticoid-like Activity of Licorice: Its Military Application by Alexander the Great. Interestingly, the main side effect of carbenoxolone is sodium retention. Carbenoxolone and licorice extract seem to act like aldosterone, the steroid hormone that promotes retention of sodium in the kidney (74). We now know that this is due to glycyrrhetinic acid and carbenoxolone inhibiting 11β-hydroxysteroid dehydrogenase in the kidney, raising the local glucocorticoid levels, which leads to a mineralocorticoid like effect: sodium retention, which in turn promotes water retention. This provides a scientific explanation for the thirst-quenching effects of licorice reported over 2000 years ago. Indeed, Alexander the Great's troops used licorice root for this purpose. They could travel long distances without water, which enabled them to move over inhospitable terrain. This is an early example of the use of medical knowledge for military purposes.

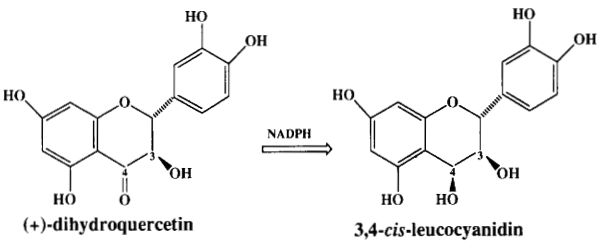
Licorice Inhibits Streptomyces Hydrogenans 20β-Hydroxysteroid Dehydrogenase. *Streptomyces hydrogenans* 20β-hydroxysteroid dehydrogenase has strong sequence similarity to 15-hydroxyprostaglandin dehydrogenase and 11β-hydroxysteroid dehydrogenase, both of which are inhibited by licorice-derived compounds. This suggested determining if licorice inhibited *S. hydrogenans* 20β-hydroxysteroid dehydrogenase. Indeed, Ghosh *et al.* found that glycyrrhizic acid and carbenoxolone inhibit this enzyme. Both compounds have μ*M* affinity for 20β-hydroxysteroid dehydrogenase (75). Thus, enzymes that have separated about 2 billion years from a common ancestor and retain sequence similarity also retain the determinants for recognizing glycyrrhizic acid.

Life in the Sun

Flower Colors, Steroid Hormone Action in Humans, and Pox Viruses. Flavonoids are precursors of anthocyanins, pigments of flowers. Conversion of a flavanone to a leuco-anthocyanidin requires sequential modification by flavoanone 3β-hydroxylase an dihydroflavonol 4-reductase. Sequence analyses show that plant dihydroflavonol 4-reductases share a common ancestor with human 3β-hydroxysteroid dehydrogenase, an enzyme that converts pregnenolone to progesterone (76, 77). The reactions catalyzed by these enzymes are shown in Figure 4. Note the similarities in



Reaction Catalyzed by Mammalian 3β-hydroxysteroid Dehydrogenase



Reaction Catalyzed by Plant Dihydroflavonol Reductase

Figure 4. Reactions catalyzed by human 3β-hydroxysteroid dehydrogenase and plant dihydroflavonol reductase.

structure of the substrates for the plant and animal enzymes.

Progesterone is a precursor for the steroids shown in Figure 1. Thus, a compound that inhibits 3β-hydroxysteroid dehydrogenase would have important endocrine effects in humans and other vertebrates. Interestingly, plant extracts that inhibit progesterone synthesis were used in ancient times as contraceptives (5).

Other analyses show that vaccinia virus, a relative of the small pox virus, contains a gene that is homologous to plant dihydroflavonol reductase, as does a cholesterol metabolizing enzyme in *Nocardia*, a soil bacterium, and *E. coli* UDP-galactose-4-epimerase. The vaccinia virus gene has 3β-hydroxysteroid dehydrogenase activity (78). If compounds that bind to dihydroflavonol reductase bind to human 3β-hydroxysteroid dehydrogenase or *E. coli* UDP-galactose-4-epimerase or one of its homologs (77), this would have profound endocrine effects on humans.

Summary

The binding of plant-derived compounds to estrogen receptors and its effect on estrogen responsive genes was established many years ago. Here we presented a model for another site of action of plant-derived compounds based on sequence homologies between enzymes in plants and bacteria and steroid and prostaglandin dehydrogenases. We propose that some of the hormone-like activity of plant-derived compounds is due to binding to animal dehydrogenases. This clearly is the case for licorice-derived compounds and may also be true for flavonoids.

1. Gibson MR. Glycyrrhiza in old and new perspectives. Lloydia **41**:348–354, 1978.
2. Monder C. Corticosteroids, kidneys, sweet roots and dirty drugs. Mol Cell Endrocrinol **78**:C95–C98, 1991.
3. Davis EA, Morris DJ. Medicinal uses of licorice through the millennia: The good and plenty of it. Mol Cell Endocrinol **78**:1–6, 1991.
4. Duke JA. Handbook of Biologically Active Phytochemicals and Their Activities. Boca Raton, FL: CRC Press, 1992.
5. Riddle JM, Estes JW. Oral contraceptives in ancient and medieval times. Am Scientist **80**:226–233, 1992.
6. Wong E, Flux DS. The oestrogenic activity of red clover isoflavones and some of their degradation products. J Endocrinol **24**:341–348, 1962.
7. Adams NR. Permanent infertility in ewes exposed to plant oestrogens. Aust Vet J **67**:197–201, 1990.
8. Markaverich BM, Roberts RR, Alejandro MA, Johnson GA, Middleitch BS, Clark JH. Bioflavonoid interaction with rat uterine type II binding sites and cell growth inhibition. J Steroid Biochem **30**:71–78, 1988.
9. Whitten PL, Naftolin F. Effects of a phytoestrogen diet on estrogen-dependent reproductive processes in immature female rats. Steroids **57**:56–61, 1992.
10. Sharma OP, Adlercreutz H, Strandberg JD, Zirkin BR, Coffey DS, Ewing LL. Soy of dietary source plays a preventive role against the pathogenesis in rats. J Steroid Biochem Mol Biol **43**:557–564, 1992.
11. Shemesh M, Linder HR, Ayalon N. Affinity of rabbit uterine oestradiol receptor for phyto-oestrogens and its use in a competitive protein-binding radioassay for plasma coumestrol. J Reprod Fertil **29**:1–9, 1972.
12. Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology **103**:1860–1867, 1978.
13. Scambia G, Ranelletti FO, Panici PB, Piantelli M, Rumi C, Battaglia F, Larocca LM, Capelli A, Mancuso S. Type-II estrogen binding sites in a lymphoblastoid cell line and growth-inhibitory effect of estrogen, anti-estrogen and bioflavonoids. Int J Cancer **46**:1112–1116, 1990.
14. Mousavi Y, Adlercreutz H. Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. Steroids **58**:301–304, 1993.
15. Pelissero C, Bennetau B, Babin P. Le Menn F, Dunogues J. The estrogenic activity of certain phytoestrogens in the siberian sturgeon *Acipenser baeri*. J Steroid Biochem Mol Biol **38**:293–299, 1991.
16. Adlercreutz H. Does fiber-rich food containing animal lignan precursors protect against both colon and breast cancer? An extension of the "fiber hypothesis". Gastroenterology **86**:761–766, 1984.
17. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M. Non-steroidal estrogens of dietary origin: Possible role in hormone-dependent disease. Am J Clin Nutr **40**:569–578, 1984.
18. Adlercreutz H, Hockerstedt K, Bannwart C, Bloigu S, Hamalainen E, Fotsis T, Ollus A. Effects of dietary components, including lignans and phyto-oestrogens, on enterohepatic circulation and liver metabolism of oestrogens and on sex hormone binding globulin (SHBG). J Steroid Biochem **27**:1135–1144, 1987.
19. Barnes S, Grubbs C, Setchell KD, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. Prog Clin Biol Res **347**:239–253, 1990.
20. Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wahala K, Makela T, Hase T. Dietary phytoestrogens and cancer: *In vitro* and *in vivo* studies. J Steroid Biochem Mol Biol **41**:331–337, 1992.
21. Adlercreutz H, Bannwart C, Wahala K, Makela T, Brunow G, Hase T, Arosemena PJ, Kellis JT, Vickery LE. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J Steroid Biochem Mol Biol **44**:147–153, 1993.
22. Peterson G, Barnes S. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. Prostate **22**:335–345, 1993.
23. Stewart PM, Valentino R, Wallace AM, Burt D, Shackleton CHL, Edwards CRW. Mineralocorticoid activity of liquorice: 11-Beta-hydroxysteroid dehydrogenase deficiency comes of age. Lancet **2**:821–823, 1987.
24. Edwards CRW, Stewarts PM, Burt D, Brett L, McIntyre MA, Sutanto WS, De Kloet ER, Monder C. Localization of 11β-hydroxysteroid dehydrogenase-tissue specific protector for the mineralocorticoid receptor. Lancet **2**:986–989, 1988.
25. Monder C, Stewart PM, Lakshmi V, Valentino R, Burt D, Edwards CR. Licorice inhibits corticosteroid 11β-dehydrogenase of rat kidney and liver: *In vivo* and *in vitro* studies. Endocrinology **125**:1046–1053, 1989.

26. Monder C. Corticosteroids, receptors, and the organ-specific functions of 11β-hydroxysteroid dehydrogenase. FASEB J **5**:3047–3054, 1991.
27. Funder JW, Pearce PT, Smith AI. Mineralocorticoid action: Target tissue specificity is enzyme, not receptor, mediated. Science **242**:583–586, 1988.
28. Funder JW, Pearce PT, Myles K, Roy LP. Apparent mineralocorticoid excess, pseudohypoaldosteronism, and urinary electrolyte excretion: toward a redefinition of mineralocorticoid action. FASEB J **4**:3234–3238, 1990.
29. Baker ME. Genealogy of regulation of human sex and adrenal function, prostaglandin action, snapdragon and petunia flower colors, antibiotics, and nitrogen fixation: Functional diversity from two ancestral dehydrogenases. Steroids **56**:354–360, 1991.
30. Degen GH. Interaction of phytoestrogens and other environmental estrogens with prostaglandin synthase *in vitro*. J Steroid Biochem **35**:473–479, 1990.
31. Baker ME, Fanestil DD. Licorice, computer-based analyses of dehydrogenase sequences and regulation of asteroid and prostaglandin action. Mol Cell Endocrinal **78**:C99–C102, 1991.
32. Baker ME, Fanestil DD. Liquorice as a regulator of steroid and prostaglandin metabolism. Lancet **337**:428–429, 1991.
33. Baker ME. Evolution of enzymatic regulation of prostaglandin action: Novel connections to regulation of human sex and adrenal function, antibiotic synthesis and nitrogen fixation. Prostaglandins **42**:391–407, 1991.
34. Baker ME. Licorice and enzymes other than 11β-hydroxysteroid dehydrogenase. Steroids **59**:136–141, 1994.
35. Long SR. Rhizobium-legume nodulation: Life together in the underground. Cell **56**:203–214, 1989.
36. Nap J-P, Bisseling T. Developmental biology of a plant-prokaryote symbiosis: The legume root nodule. Science **250**:948–954, 1990.
37. Fisher RF, Long SR. Rhizobium-plant signal exchange. Nature **357**:655–660, 1992.
38. Gyorgypal Z, Kiss GB, Kondorosi A. Transduction of plant signal molecules by the *Rhizobium* NodD proteins. Bioessays **11**:575–581, 1991.
39. Gabriel DW, Rolfe BG. Working models of specific recognition in plant-microbe interactions. Annu Rev Phytopathol **28**:365–391, 1990.
40. Stafford HA. Flavonoid evolution—an enzyme approach. Plant Physiol **96**:680–685, 1991.
41. Koes RE, Quattrocchio F, Mol JNM. The flavonoid biosynthetic pathway in plants: Function and evolution. BioEssay **16**:123–132, 1994.
42. Baker ME. Similarities between legume-rhizobium communication and steroid-mediated intercellular communication in vertebrates. Can J Microbiol **38**:541–547, 1992.
43. Baker ME. Evolution of regulation of steroid-mediated intercellular communication in vertebrates: Insights from flavonoids, signals that mediate plant-rhizobia symbiosis. J Steroid Biochem Mol Biol **41**:301–308, 1992.
44. Uchida S, Nonoguchi H, Endou H. Localization and properties of NAD+-dependent 15-hydroxyprostaglandin dehydrogenase activity in the rat kidney. Pflugers Arch **404**:278–284, 1985.
45. Williams WM, Frolich JC, Nies AS, Oates JA. Urinary prostaglandins: Site of entry into renal tubular fluid. Kidney Int **11**:256–260, 1977.
46. Bonvalet JP, Pradelles P, Farman N. Segmental synthesis and actions of prostaglandins along the nephron. Am J Physiol **253**:F377–F387, 1987.
47. Hopwood DA, Sherman DH. Molecular genetics of polyketides and its comparison to fatty acid biosynthesis. Annu Rev Genet **24**:37–66, 1990.
48. Robinson JA. Polyketide synthetase complexes: Their structure and function in antibiotic synthesis. Phil Trans R Soc London B Biol Sci **332**:107–114, 1991.
49. Chadwick DJ, Whelan J, Eds. Secondary Metabolites: Their Function and Evolution. Ciba Foundation Symposium. New York: Wiley, 171, 1992.
50. Baker ME. A common ancestor for human placental 17β-hydroxysteroid dehydrogenase, *Streptomyces coelicolor* actIII protein, and *Drosophila melanogaster* alcohol dehydrogenase. FASEB J **4**:222–226, 1990.
51. Davies J. What are antibiotics? Archaic functions for modern activities. Mol Microbiol **4**:1227–1232, 1990.
53. Stone MJ, Williams DH. On the evolution of functional secondary metabolites (natural products). Mol Microbiol **6**:29–34, 1992.
54. Lamb CJ, Lawton MA, Dron M, Dixon RA. Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell **56**:215–224, 1989.
55. Lyttle CR, Medlock KL, Sheehan DM. Eosinophils as the source of uterine nuclear type II estrogen binding sites. J Biol Chem **259**:2697–2700, 1984.
56. Malloy PJ, Zhao X, Madani ND, Feldman D. Cloning and expression of the gene from *Candida albicans* that encodes a high-affinity corticosteroid-binding protein. Proc Natl Acad Sci USA **90**:1902–1906, 1993.
57. Baker ME. Human placental 17β-hydroxysteroid dehydrogenase is homologous to NodG protein of *Rhizobium meliloti*. Mol Endocrinol **3**:881–884, 1989.
58. Baker ME. Protochlorophyllide reductase is homologous to human carbonyl reductase and pig 20β-hydroxysteroid dehydrogenase. Biochem J **300**:605–607, 1994.
59. Tannin GM, Agarwal AK, Monder C, New MI, White PC. The human gene for 11β-hydroxysteroid dehydrogenase. J Biol Chem **266**:16653–16658, 1991.
60. Persson B, Krook M, Jornvall H. Characteristics of short-chain alcohol dehydrogenases and related enzymes. Eur J Biochem **200**:537–543, 1991.
61. Krozowski Z. 11β-Hydroxysteroid dehydrogenase and the short chain alcohol dehydrogenase (SCAD) superfamily. Mol Cell Endocrinol **84**:C25–C31, 1992.
62. DeLong A, Calceron-Urrea A, Dellaporta SL. Sex determination gene *TASSELSEED2* of maize encodes a short-chain-alcohol dehydrogenase required for stage-specific floral organ abortion. Cell **74**:757–768, 1993.
63. Leenders F, Adamski J, Husen B, Thole HH, Jungblut PW. Molecular cloning and amino acid sequence of the porcine 17β-estradiol dehydrogenase. Eur J Biochem **222**:221–227, 1994.
64. Baker ME. A common ancestor for Candida tropicalis and dehydrogenases that synthesize antibiotics and steroids. FASEB J **4**:3028–3032, 1990.
65. Skowronski R, Feldman D. Characterization of an estrogen-binding protein in the yeast *Candida albicans*. Endocrinology **124**:1965–1972, 1989.
66. Powell BL, Frey CL, Drutz DJ. Identification of a 17β-estradiol biding protein in *Candida albicans* and *Candida* (Torulopiss) *glabrata*.Exp Mycol **8**:304–307, 1984.
67. Burshell A, Stathis PA, Do Y, Miller SC, Feldman D. Characterization of an estrogen-binding protein in the yeast *Saccharomyces cerevisiae*. J Biol Chem **259**:3450–3456, 1984.
68. Reevers FE. De behandeling von ulcus ventriculi en ulcus duodenei met succcus liquiritieae. Ned Tijdschr Geneesk **92**:2968–2973, 1948.
69. Jones FA. General introduction. In: Robson JM, Sullivan FM, Eds. A Symposium on Carbenoxolone Sodium. London: Butterworths, pp1–4, 1968.
70. Borst JGG, Ten Holt SP, De Vries LA, Molhuysen JA. Synergistic action of liquorice and cortisone in Addison's and Simmonds's disease. Lancet **2**:657–663, 1953.

71. Brown HM, Christie BGB, Colin-Jones E, Finney RSH, MacGregor WG, Smith JM, Smith JM, Smith WG, Tarnoky AL, Turner EE, Wotton DEM, Watkinson G. Glycyrrhetinic acid hydrogen succinate (disodium salt): A new anti-inflammatory compound. Lancet **2**:492–493, 1959.
72. Pinder RM, Brogden RN, Sawyer PR, Speight TM, Spencer R, Avery GS. Carbenoxolone: A review of its pharmacological properties and therapeutic efficacy in peptic ulcer disease Drugs **11**:245–307, 1976.
73. Doll R, Hill ID, Hutton C, Underwood DJ II. Clinical trial of a triterpenoid liquorice compound in gastric and duodenal ulcer. Lancet **2**:793–796, 1962.
74. Card WI, Mitchell W, Strong JA, Taylor NRW, Tompsett SL, Wilson JMG. Effects of liquorice and its derivatives on salt and water metabolism Lancet **1**:663–667, 1953.
75. Ghosh D, Erman M, Pangborn W, Duax W, Baker ME. Inhibition of *Streptomyces hydrogenans* 3α,20β-hydroxysteroid dehydrogenase by licorice-derived compounds and crystallization of an enzyme-cofactor-inhibitor complex. J Steroid Biochem Mol Biol **42**:849–853, 1992.
76. Baker ME, Luu-The V, Simard J, Labrie F. A common ancestor for mammalian 3β-hydroxysteroid dehydrogenase and plant dihydroflavonol reductase. Biochem J **269**:558–558, 1990.
77. Baker ME, Blasco R. Expansion of the mammalian 3β-hydroxysteroid dehydrogenase/plant dihydroflavonol reductase superfamily to include a bacterial cholesterol dehydrogenase, a bacterial UDP-galactose-4-epimerase, and open reading frames in vaccinia virus and fish lymphocystis disease virus. FEBS Lett **301**:89–93, 1992.
78. Moore JB, Smith GL. Steroid hormone synthesis by a vaccinia enzyme-a new type of virus virulence factor. EMBO J **11**:1973–1980, 1992.