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Naturally-Occurring Estrogens in Plant Foodstuffs - A Review

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ABSTRACT

A number of non-steroidal estrogenic substances are common naturally-occurring constituents of human foods. Concern over dietary estrogens has focused largely on the consumption of trace amounts of diethylstilbestrol (DES) from tissues of cattle fed the compound as a growth stimulant. Human exposure to naturally-occurring fungal and phytoestrogens in foods is, however, substantially larger than exposure to DES in animal tissues. Occurrence, potency and toxicity of the estrogenic isoflavones, coumestans and resorcylic acid lactones are reviewed.

Numerous chemicals which are tumorigenic or carcinogenic in experimental animals are common naturally-occurring constituents of foods. Nitrosamines, aflatoxins, polycyclic aromatic hydrocarbons and estrogens can all be shown, under appropriate conditions, to be carcinogens and are all commonly consumed in foods. The significance of these compounds in the etiology of various human cancers is, of course, the subject of considerable controversy. The possible significance of naturally-occurring estrogens in foods has been widely publicized in recent years by the public debate and scientific discussion over the presence of diethylstilbestrol (DES) residues in liver or other tissues from animals treated with DES as a growth stimulant. DES is widely used in production of cattle in the U.S. although its use was at one time banned by the FDA (*1,2*). Amounts of DES present in meat and poultry products which led to the ban were extremely small and it has been frequently suggested that the dietary estrogens to which humans are exposed are predominantly naturally-occurring phytoestrogens rather than residues of feed additives. The significance of this comparison of intake levels is, of course, complicated both by problems in applying dose-response toxicology to carcinogenesis and by the question of whether more stringent standards should apply to regulation of intentional additives than apply to regulation of naturally-occurring substances of a similar kind. Resolution of these issues is beyond the scope of this review. The intent here is to summarize what is known about naturally-occurring non-steroidal estrogens in plants and plant foodstuffs.

ESTROGENS IN PLANTS

Existence of estrogenic substances in plants has been recognized for a considerable time. Bradbury and White in 1954 listed over 50 species of plants which had been shown to possess estrogenic activity and many more have been reported since that time (*15,23,24*). As shown by data in Table 1, estrogenic activity has been detected in a wide variety of food products, some of which are of major importance in our food supply. In most instances the chemical constituents responsible for the estrogenic activity of these plants have not been characterized. Reports of estrogenic activity are based most commonly on either evidence of uterine enlargement or of cornification of vaginal epithelium after treatment of experimental animals with plant extracts. Both of these assays are subject to several criticisms and misleading results are possible (*22*). The effect of the physiological state of the plant has only rarely been considered as an important variable in estrogenicity studies. The estrogen levels in clover are, for example, known to be affected by conditions of growth, climate, variety, stage of growth and other similar parameters (*28*). Variability in the reported estrogenicity of hops (Table 1) may be due to effects of this kind.

TABLE 1. *Estrogenic activity of selected plant foodstuffs.*

Source	Amount	Reference
Carrots, fresh	+	*26*
Cabbage	0.024 μg E_2/g[a]	*19*
Peas	0.004-0.006 μg E_2/g	*19*
Hops	1-2 μg E_2/g	*19*
	20-300 μg E_2/g	*40*
	None	*25*
Wheat bran	+	*12*
Wheat germ	+	*12*
Rice bran	+	*12*
Rice polish	+	*12*
Soybean meal	+	*55,56*
Vegetable oils	+	*12,69*
Pomegranate seeds	4 μg estrone/kg	*21*
	17 mg estrone/kg	*29*
Milk	+	*66*

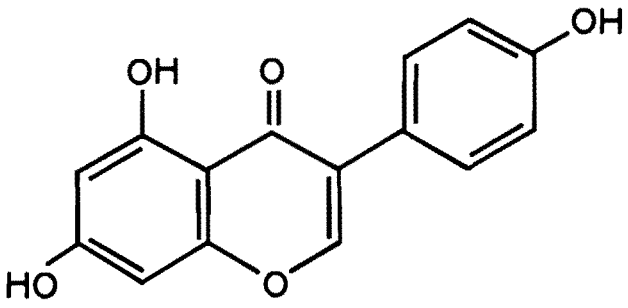
[a] E_2 = 17-β-estradiol.

It is somewhat unfortunate for our purposes that most of the information available on plant estrogens is concerned with estrogenic substances in feeds (clover, alfalfa, fungus-infected grains), rather than concerned specifically with those estrogens of significance in human foods. Nevertheless, most of the information obtained from these feed-directed studies is pertinent and will be discussed in this review of the literature. Those phytoestrogens which have been characterized fit into three general chemical categories: isoflavones, coumestans and resorcylic acid lactones. It should be

emphasized, however, that many other classes of compounds to which man is commonly exposed may possess either uterotropic or estrogenic properties. Gibberellic acid, for example, is a widely distributed plant hormone which has been reported to possess estrogenic properties (*45,46*). Several insecticides have estrogen-like activity (*63*). The estrogenicity of delta-9-tetrahydrocannabinol has been recently debated (*60,76*).

ISOFLAVONES

Isoflavones are the most common naturally-occurring isoflavonoids. Wong (*85*) listed over 70 isoflavones and 40 isoflavone glycosides which have been shown to occur naturally. Many of the compounds which have been isolated as free isoflavones may exist in vivo as glucosides. (*4,80*). Only a limited number of these compounds have been shown to be estrogenic. Genistein (4',5,7-trihydroxyisoflavone) was first isolated from subterranean clover and, though not a steroid, has a structure with



GENISTEIN

some resemblance to that of estradiol and diethylstilbestrol. Genistein stimulates uterine growth in ovariectomized mice (*5,86*), sheep (*16*), and rats (*59*). In mice genistein is roughly 10^5 times less effective than diethylstilbestrol as an estrogen in stimulating uterine enlargement (Table 2) (*6,16*). Based on competitive binding to human tumor cell estradiol receptors, however, genistein is only 50 times less potent than 17β-estradiol (Table 3). Clearly, affinity for estradiol receptors indicates a much higher potency than is suggested by in vivo uterine weight assays. It is probable that transport and metabolic effects are responsible for this difference. It is not clear at this time which method of estimating estrogenicity is of most value in predicting potency in humans.

TABLE 2. *Relative potency of some naturally-occurring estrogens in mice.*[a]

	Quantity to produce 25 mg uterus (μg)	Relative potency
Diethylstilbestrol	0.083	100.000
Estrone	1.2	6.900
Coumestrol	240	35
Coumestroldiacetate	340	24
Genistein	8,000	1.0[b]
Daidzein	11,000	0.75
Biochanin A	18,000	0.46
Formononetin	32,000	0.26

[a]From (*6*).
[b]Genistein arbitrarily assigned a value of 1.0.

Other common isoflavones which have been shown to be estrogenic are daidzein (4',7-dihydroxyisoflavone), biochanin A (5,7-dihydroxy-4'-methoxy-isoflavone), and formononetin (7-hydroxy-4'-methoxyisoflavone). Based on either relative affinity for estrogen receptors or assays using mouse uterine weight these compounds are very weak estrogens (Table 2, Fig. 1). Other classes of flavonoids have been examined for estrogenic activity but appear to be relatively inactive compared to the isoflavones.

Investigations of the estrogenic isoflavones began in the 1940s as an attempt to explain the causes of "clover disease" which resulted in infertility in sheep grazing on certain forages. The problem was traced to the presence of estrogenic isoflavones in several common clovers. Concentrations of isoflavones in clover can be as great as 5% of the dry matter content of the leaves of healthy plants (*73*). Genistein and formononetin are the major isoflavones present in clovers responsible for reproductive problems in sheep. Because formononetin is a relatively weak estrogen in mice, whereas genistein is quite active in mice and poultry (*18*) and in guinea pigs (*54*), genistein was thought for some time to be the principal cause of 'clover disease' in sheep. However, genistein content of clover varieties does not correlate well with estrogenic activity in sheep, whereas formononetin content correlates quite well (*50*). The high estrogenic activity in sheep of formononetin compared to genistein has been shown to be a direct result of the metabolism of the two isoflavones. Genistein and

TABLE 3. *Relative affinity of phytoestrogens for mammalian estrogen receptors.*

	Receptor			
Estrogen	Rabbit uterine cytosol (*71*)	Sheep uterine cytosol (*74*)	Human cancer cell line MCF-7 (*47*)	Rat uterine cytosol (*84*)
17β-estradiol	100	100	100	100
HMP zearalanol	—	—	20	21.3
Coumestrol	1.4	5	10	5.0
Zearalenone	—	—	3.3	3.4
Genistein	0.6	0.9	2	1.3
LMP zearalanol	—	—	1.3	0.6
Daidzein	—	0.1	—	0.09
Formononetin	—	<0.01	<0.01	—
Biochanin A	—	—	—	0.07

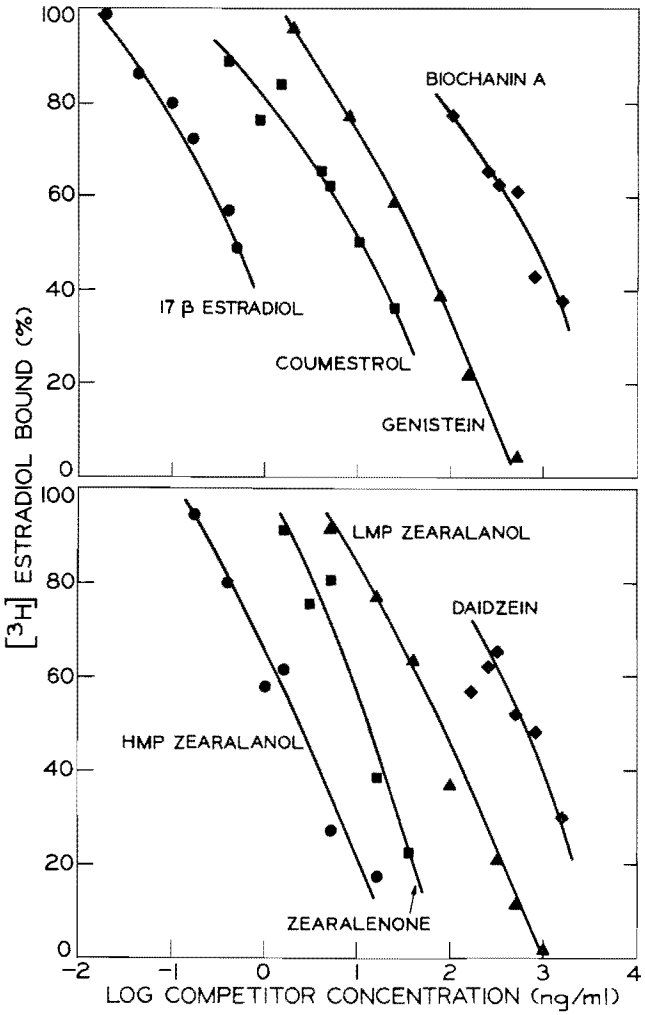


Figure 1. *Competitive binding of plant and fungal estrogens to ^3H-estradiol binding proteins from rat uterine cytosol. From Verdeal* (84).

biochanin A are degraded (Fig. 2) in the rumen to give p-ethyl-phenol and a phenolic acid, neither of which are estrogenic. The primary pathway for degradation of formononetin, however, is demethylation to give daidzein and subsequent reduction to give equol, an estrogenically active compound (*3,75*). The relative estrogenicity of genistein and formononetin in sheep is therefore the opposite of the situation in mice.

The pathway for degradation of genistein and biochanin A appears to be induced in sheep only after several days of ingestion of feed rich in isoflavones. In contrast, metabolism of formononetin to equol does not change significantly with time. The estrogenicity of genistein and biochanin A, then, appears to depend on the feeding history of the animal while the estrogenicity of formononetin does not exhibit such a dependence (*20,73*). The ability of an animal to develop metabolic pathways to inactivate estrogenic isoflavones, then, can have a decisive effect on the overall significance of the presence of these substances in foods. It is extremely difficult for this reason to draw conclusions about a compound's estrogenicity in one species from experiments with another species. Unfortunately very little is known about the metabolism of estrogenic isoflavones in nonruminants in general and in man in particular (*41*).

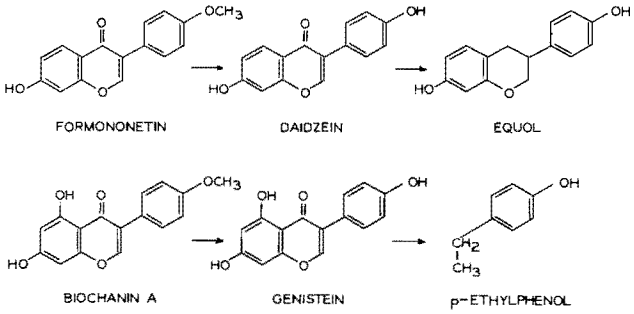
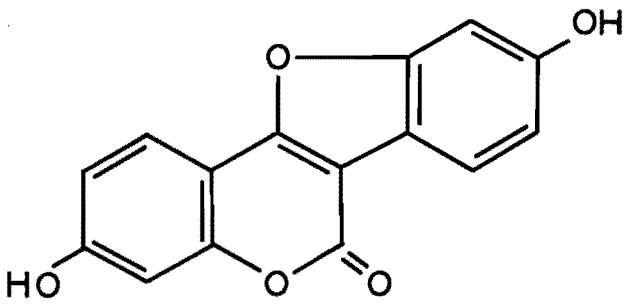


Figure 2. *Metabolism of estrogenic isoflavones in the rumen of the sheep. Adapted from Shutt* (73).

Several isoflavones appear to possess antifungal or antibacterial properties and coordinate derepression of isoflavonoid biosynthesis may be responsible in part for the hypersensitive resistant response of plant tissue to invasion by pathogens. For example, daidzein accumulates to high levels in soybeans after infection with either *Phytophthora megasperma* or *Phytophthora glycinea* (*36,38*). Daidzein also accumulates in ozone-injured soybean leaves (*37*). Other isoflavones which have been shown to accumulate after fungal infection of various plants include genistein (*10*), 2',4',5,7 tetrahydroxyisoflavone (*10*) and formononetin (*61*). Free isoflavones have been shown to have higher fungistatic activity than the corresponding glycosides (*55*) and a glycosidase from the invading fungus may be responsible for activation of the isoflavonoids synthesized by the plant (*62*). Biosynthesis of isoflavones in response to physical damage or fungal infection could be one reason for variations in isoflavone content of foods.

COUMESTANS

A second class of isoflavonoids that contains compounds which possess estrogenic activity is the coumestans (coumaranocoumarins). Over 20 naturally-occurring coumestans have been reported (*85*). The coumestans which have been most thoroughly studied are the estrogenic coumestans from alfalfa (*Medicago sativa*) and ladino clover (*Trifolium repens*) (Table 4). The dominant estrogen in alfalfa appears to be coumestrol (*9*).



COUMESTROL

TABLE 4. *Naturally-occurring coumestans isolated from alfalfa.*

Common name	Trivial name	Reference
Coumestrol	7,12-Dihydroxy-coumestan	*5*
Trifoliol	7,10-Dihydroxy-12-methoxy-coumestan	*42*
Medicagol	7-Hydroxy-11,12-methylene-dioxycoumestan	*43*
4'-Methoxycoumestrol	7,12-Dihydroxy-12-methoxy-coumestan	*7*
3'-Methoxycoumestrol	7,12-Dihydroxy-11-methoxy-coumestan	*8*
Lucernol	6,7,12-Trihydroxycoumestan	*77*
Sativol	7-Methoxy-8,12-dihydroxy-coumestan	*77*
—	7-Hydroxy-11,12-dimethoxy-coumestan	*78*

Coumestrol has been detected in several clovers as well as in many other plant products more commonly consumed by man (Table 5). Based on the dosage required to produce a uterine weight of 25 mg in mice, coumestrol has been estimated to be 30-100 times more active as an estrogen than are the isoflavones (Table 2). The affinity of coumestrol for mammalian estradiol receptors is only roughly 10 to 20 times lower than the affinity of 17β estradiol (Table 3) (Fig. 1). Coumestrol binds to high-affinity estradiol-binding proteins in DMBA-induced rat mammary tumor tissue and in human mammary tumor tissue (*84*).

TABLE 5. *Coumestrol content of plant products.*[a]

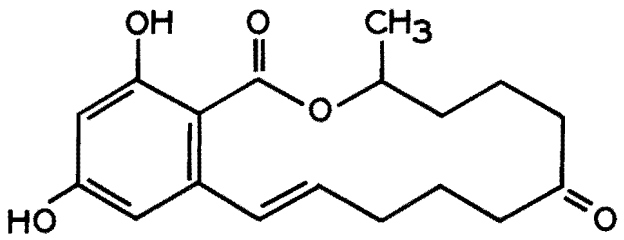
Product	Coumestrol, μg/g dry weight
Alfalfa sprouts (fresh)	5.0
Soybean sprouts (fresh)	71.1
Soybean (dry)	1.2
Soybean meal defatted (dry)	0.4
Soybean concentrate (dry)	0.2
Soybean isolate (dry)	0.6
Green beans (frozen)	1.0
Snow peas (frozen)	0.6
Green peas (frozen)	0.4
Brussell sprouts (frozen)	0.4
Red beans (dry)	0.4
Split peas (dry)	0.3
Spinach leaf (frozen)	0.1

[a]From (*39*).

As was true with the isoflavones, the coumestrol content of clover and other plants is influenced by conditions of growth, climate, varietal and genetic differences and stage of growth (*28*). Germination of soybeans appears to increase coumestrol content by a factor of nearly 60 (Table 5). A considerable amount of evidence has accumulated which demonstrates that synthesis of coumestrol in plants is affected by disease or insect infestation. For example, accumulation of coumestrol occurs in hypocotyls of soybeans infected with a fungus (*38*) and has been associated with the hypersensitive resistance response in lima beans (*65*). White clover does not normally exhibit any estrogenic activity, but after fungal infection it can produce estrogenic effects in sheep (*57*). Concentrations of coumestrol as high as 2362 ppm have been reported in leaves of severely infected plants (*44*). High coumestrol contents of alfalfa extracts may be a problem in use of alfalfa leaf protein concentrate on a large scale (*39*).

RESORCYLIC ACID LACTONES

The third class of estrogenic substances which occur in plant foodstuffs are derivatives of resorcylic acid lactones. Zearalenone [6 (10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid lactone] and its derivatives (Table 6) are mycotoxins synthesized by the mold



ZEARALENONE

TABLE 6. *Naturally-occurring derivatives of zearalenone.*

Name	Reference
8'hydroxyzearalenone	*35*
8'-epi-hydroxyzearalenone	*35*
5 formylzearalenone	*11*
7'Dehydrozearalenone	*11*
6'8'Dihydroxyzearalenone	*51*

Fusarium roseum which can infect corn, wheat, barley, sorghum or hay (*33,51,53,72*). Zearalenone has been referred to as "fermentation estrogenic substance (FES)" or as F-2 toxin. *Fusarium* infection is very common. Surveys of the U.S. corn and wheat crops show a 10-20% incidence of zearalenone contamination of marketable corn and wheat with contamination as high as 10 ppm zearalenone (*30,79*). High concentrations of zearalenone occur generally only after storage of infected grains; however, zearalenone has been identified in freshly harvested corn (*17*). A derivative of zearalenone, zearalanol, has been patented for use as a growth stimulant in animals by Commercial Solvents Corporation under the trade name Ralgro. Its use as an ear implant in cattle has been approved by the FDA (*13,64,70*).

Numerous effects of zearalenone in experimental animals and in model systems have been observed. Swine, which are particularly sensitive to zearalenone, develop several reproductive disorders on diets containing *Fusarium*-infected feeds. Approximately 1-5 ppm zearalenone (4 mg cumulative total dose) is sufficient to cause signs of vulvovaginitis in gilts (*51*). Miller and co-workers have shown that sows and gilts receiving 5 mg of purified zearalenone daily throughout the last month of pregnancy produced litters with stillborn pigs or pigs with a "spayleg" incoordination of hind limbs (*49*). When administered subcutaneously, zearalenone is less active in the assay for mouse uterine enlargement than when administered by gavage, suggesting that some

metabolic intermediate more active than zearalenone may be involved (*52*). Both zearalenone and zearalanol bind to estradiol-binding sites from several mammalian tissues (Table 3) (Fig. 1) (*14,27,47,84*). Zearalenone has been suggested to be mutagenic by Ueno and Kubota (*81*) who used a recombination-deficient mutant cell line of *Bacillus subtilis*. The cytotoxic effect of zearalenone in various monolayer cell cultures has been investigated (*83*). Dietary zearalenone has also been suggested as a possible cause of spontaneous tumors in laboratory animals (*38,67*) although no solid evidence to support this suggestion has been provided. Hobson et al. have examined the effects of zearalenone on serum gonadotropins in ovariectomized rhesus monkeys and shown that zearalenone is only slightly less potent than estradiol or DES when administered by subcutaneous injection but is substantially less potent when administered orally (*34*).

In addition to the numerous effects which have been observed in experimental animals and in model systems, zearalenone appears to be estrogenic in humans. At a dose of 75-100 mg/day zearalenone appears to be effective in treatment of symptoms of postmenopausal syndrome in women (*31,82*). Both zearalanol and zearalenone have been reported to be effective oral contraceptive agents in humans and have been patented for this use (*32*). Zearalenone and its derivatives are the only naturally-occurring non-steroidal estrogens which have been shown to be estrogenic in vivo in man. Amounts of zearalenone to which humans are likely to be exposed in foods are several orders of magnitude lower than the amounts necessary for contraception. The significance of low-level long-term exposure to these compounds either in the etiology of hormone-dependent tumors or in the responsiveness of hormone-dependent tumors to hormone therapy is not known.

HUMAN EXPOSURE TO DIETARY ESTROGENS

Table 7 lists estimates of the extent of human exposure to estrogens of various kinds measured in DES equivalents. Examples of exposure to phytoestrogens given in this table are on the basis of quantitative analysis of specific phytoestrogens. Estimates of total estrogen content of plant foods could be considerably higher. Unless phytoestrogens are metabolized to more potent estrogens, however, it is clearly not likely that humans are exposed to dietary doses sufficient to cause any major physiological response. The possibility of metabolic alteration to more or less active forms should not be ignored, however, since effects of this kind have been demonstrated in experimental animals. Likely human exposure to phytoestrogens when measured in DES equivalents is considerably higher than likely human exposure to DES in liver from cattle treated with DES as a growth stimulant. This is particularly true since 0.5 ppb DES in liver is a large overestimate of actual DES levels and would only occur in liver from improperly treated cattle. It seems probable, then, that actual human exposure to phytoestrogens is substantially higher than human exposure to DES residues.

TABLE 7. *Human exposure to exogenous estrogens.*

Source	Estimate of possible daily dose (μg /DES quivalents)[a]	Reference
Morning-after pill	50,000	*2*
Birth control pill	2,500	*2*
Post-hysterectomy replacement therapy	500-1,000	*2*
Post-menopausal therapy	500	*2*
100 g beef liver with 0.5 ppb DES	0.05	*2*
100 g wheat with 2 ppm zearalenone	0.2	*30*
20 g (dry weight) soybean sprouts with 70 ppm coumestrol	0.5	*39*
100 g beans (P. vulgaris) with 2-10 ppb estradiol	0.03-0.15	*87*

[a]Based on relative potencies shown in Table 2, a relative potency of 100 for zearalenone and the assumption that the oral potency of estradiol is 15% that of DES.

Considerable controversy exists over the significance of long-term dietary exposure to amounts of estrogen smaller than physiological or pharmacological doses. Relatively large doses of estrogens can induce mammary tumors to develop de novo (*58*) but can also inhibit growth of some established tumors (*48*). Smaller amounts in the range of physiological doses of estrogen, however, can stimulate tumor growth. Since human exposure to dietary estrogens is below physiological levels, resolution of questions about the importance of dietary estrogens in the etiology of human cancers must in all probability await the resolution of more general questions relating to the applicability of dose-response considerations to carcinogenesis.

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REFERENCES

1. Anonymous. 1973. Diethylstilbestrol-order denying a hearing and withdrawing approval of new animal drug applications for diethylstilbestrol implants. Fed. Register 38:10485.
2. Anonymous. 1977. Hormonally active substances in foods: a safety evaluation. Council for Agricultural Science and Technology, Report No. 66.
3. Batterham, T. J., D. A. Shutt, N. K. Hart, A. W. H. Braden, and H. J. Tweeddale. 1971. Metabolism of intraruminally administered [4-^{14}C]formononetin and [4-^{14}C]biochanin A in sheep. Aust. J. Agric. Res. 22:131-138.
4. Beck, A. B., and J. R. Knox. 1971. The acylated isoflavone glycosides from subterranean clover and red clover. Aust. J. Chem. 24:1509-1518.
5. Bickoff, E. M., A. N. Booth, R. L. Lyman, A. L. Livingston, C. R. Thompson, and F. DeEds. 1957. Coumestrol, a new estrogen isolated from forage crops. Science 126:969-970.
6. Bickoff, E. M., A. L. Livingston, A. P. Hendrickson, and A. N. Booth. 1962. Relative potencies of several estrogen-like compounds found in forages. J. Agr. Food Chem. 10:410-412.
7. Bickoff, E. M., A. L. Livingston, S. C. Witt, R. E. Lundin, and R. R. Spencer. 1965. Isolation of 4'-O-methylcoumestrol from alfalfa. J. Agr. Food Chem. 13:597-599.
8. Bickoff, E. M., R. R. Spencer, B. E. Knuckles, and R. E. Lundin. 1966. 3'-Methoxycoumestrol from alfalfa: isolation and character-

ization. J. Agr. Food Chem. 14:444-446.
9. Bickoff, E. M., R. R. Spencer, S. C. Witt, and B. E. Knuckles. 1969. Studies on the chemical and biological properties of coumestrol and related compounds. USDA Technical Bulletin No. 1408.
10. Biggs, R. 1975. Post-infectional compounds from the French bean, *Phaseolus vulgaris*. Isolation and identification of genistein and 2'4'57 tetrahydroxy-isoflavone. Aust. J. Chem. 28:1389-1392.
11. Bolliger, G., and C. Tamm. 1972. Four new metabolites from *Gibberella zeae*: 5-Formylzearalenone, 7'-dehydrozearalenone, 8'-hydroxy and 8'-epi-hydroxy-zearalenone. Helv. Chim. Acta 55:3030-3048.
12. Booth, A. N., E. M. Bickoff, and G. O. Kohler. 1960. Estrogen-like activity in vegetable oils and mill by-products. Science 131:1807-1808.
13. Borger, M. L., L. L. Wilson, J. D. Sink, J. H. Ziegler, and S. L. Davis. 1973. Zeranol and dietary protein level effects on live performance, carcass merit, certain endocrine factors and blood metabolite levels in steers. J. Anim. Sci. 36:706-711.
14. Boyd, P. A., and J. L. Wittliff. 1978. Mechanism of fusarium mycotoxin action in mammary gland. J. Toxicol. Environ. Health 4:1.
15. Bradbury, R. B., and D. E. White. 1954. Estrogens and related substances in plants. Vitamins Hormones 12:207-233.
16. Braden, A. W. H., N. K. Hart, and J. A. Lamberton. 1967. The oestrogenic activity and metabolism of certain isoflavones in sheep. Aust. J. Agr. Res. 18:335-348.
17. Caldwell, R. W., and J. Tuite. 1974. Zearalenone in freshly harvested corn. Phytopathology 64:752-753.
18. Cayen, M. N., G. Tang, and R. G. Common. 1965. Urinary conversion products of biochanin A and formononetin in the fowl. Biochim. Biophys. Acta 111:349-357.
19. Chury, J. 1960. Veber den Phytoostrogengehalt einiger Pflanzen. Experientia 16:194-195.
20. Cox, R. I., and A. W. Braden. 1974. The metabolism and physiological effects of phytoestrogens in livestock. Proc. Aust. Soc. Anim. Prod. 10:122-129.
21. Dean, P. O. G., D. Exley, and T. W. Goodwin. 1971. Steroid estrogens in plants — reestimation of estrone in pomegranate D seeds. Phytochemistry 10:2215-2216.
22. Emmens, C. W. 1969. Estrogen. pp. 62-121. In R. J. Dorfman (ed.) Methods in hormone research, Vol. IIA, (2nd ed.), Academic Press, New York, N. Y.
23. Farnsworth, N. R., A. S. Bingel, G. A. Cordell, F. A. Crane, and H. H. S. Fong. 1975. Potential value of plants as sources of new antifertility agents. I. J. Pharm. Sci. 64:535-598.
24. Farnsworth, N. R., A. S. Bingel, G. A. Cordell, F. A. Crane, and H. H. S. Fong. 1975. Potential value of plants as sources of new antifertility agents II. J. Pharm. Sci. 64:717-754.
25. Fenselau, C., and P. Talalay. 1973. Isoestrogenic activity present in hops? Food Cosmet. Toxicol. 11:597-693.
26. Ferrando, R., M. M. Guilleux, and A. Guerillot-Vinet. 1961. Oestrogen content of plants as a function of conditions of culture. Nature 192:1205.
27. Greenman, D. L., J. L. Wittliff, P. A. Boyd, and R. G. Mehta. 1977. Interaction of fusarium mycotoxins with estradiol binding sites in target tissues. J. Toxicol. Environ. Health 3:348-349.
28. Hanson, C. H., G. M. Loper, G. O. Kohler, E. M. Bickoff, K. W. Taylor, W. R. Kehr, E. H. Stanford, J. W. Dudley, M. W. Pederson, E. L. Sorensen, H. L. Carnahan, and E. P. Wilsie. 1965. Variation in coumestrol content of alfalfa as related to location, variety, butting, year, stage of growth and disease. USDA Tech. Bull. 1333.
29. Heftman, E., S.-T. Ko, and R. D. Bennett. 1966. Identification of estrone in pomegranate seeds. Phytochemistry 5:1337-1339.
30. Hesseltine, C. W., R. F. Rogers, and O. Shotwell. 1978. Fungi, especially *Gibberella zeae*, and zearalenone occurrence in wheat. Mycologia 70:14-18.
31. Hidy, P. H., and R. S. Baldwin. 1976. Composition and method. U.S. Pat. 3,965,275. June 22.
32. Hidy, P. H., and R. S. Baldwin. 1976. Method of preventing pregnancy with lactone derivatives. U.S. Pat. 3,965,274. June 22.
33. Hidy, P. H., R. S. Baldwin, R. L. Greasham, C. L. Keith, and J. R. McMullen. 1977. Zearalenone and some derivatives: production and biological activities. Adv. Appl. Microbiol. 22:59-82.
34. Hobson, W., J. Bailey, and G. B. Fuller. 1977. Hormone effects of zearalenone in nonhuman primates. J. Toxicol. Environ. Health 3:43-57.
35. Jackson, R. A., S. W. Fenton, C. J. Mirocha, and G. Davis. 1974. Characterization of two isomers of 8'-hydroxyzearalenone and other derivatives of zearalenone. J. Agr. Food Chem. 22:1015-1019.
36. Keen, N. T., and B. W. Kennedy. 1974. Hydroxyphaseollin and related isoflavonoids in the hypersensitive resistance reaction of soybeans to pseudomonasglycinea. Physiol. Plant Pathol. 4: 173-185.
37. Keen, N. T., and O. C. Taylor. 1975. Ozone injury in soybeans, isoflavonoid accumulation is related to necrosis. Plant Physiol. 55: 731-733.
38. Keen, N. T., A. J. Zaki, and J. J. Sims. 1972. Biosynthesis of hydroxyphaseollin and related isoflavonoids in disease-resistant soybean hypocotyls. Phytochemistry 11:1031-1039.
39. Knuckles, B. E., D. deFremery, and G. O. Kohler. 1976. Coumestrol content of fractions obtained during wet processing of alfalfa. J. Agric. Food Chem. 24:1177-1180.
40. Koch, W., and G. Heim. 1953. Ostrogene Hormone in Hopfen und Bier. Münch. Med. Wschr. 95:845.
41. LaBow, R. S., and D. S. Layne. 1972. The formation of glucosides of isoflavones and of some other phenols by rabbit liver microsomal fractions. Biochem. J. 128:491-497.
42. Livingston, A. L., E. M. Bickoff, R. E. Lundin, and L. Jurd. 1964. Trifoliol, a new coumestan from Ladino clover. Tetrahedron 20: 1963-1970.
43. Livingston, A. L., S. C. Witt, R. E. Lundin, and E. M. Bickoff. 1965. Medicagol, a new coumestan from alfalfa. J. Org. Chem. 30: 2353-2355.
44. Loper, G. M. 1968. Accumulation of coumestrol in barrel medic (*Medicago littoralis*). Crop Sci. 8:317-319.
45. Maillet, M., and C. Bouton. 1969. Demonstration of an estrogenic effect of gibberellic acid on the uterus of an adult castrated female rat. Therapie 24:497-508.
46. Maillet, M., C. Bouton, and M. J. Feintuch. 1969. Hormonal and tropic action of gibberellic acid in the castrated adult rat. Rev. Roum. Endocrinol. 6:279-286.
47. Martin, P. M., K. B. Horowitz, D. S. Ryan, and W. L. McGuire. 1978. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. Endocrinology (In press).
48. Meites, J., E. Cassell, and J. Clark. 1971. Estrogen inhibition of mammary tumor growth in rats; counteraction by prolactin. Proc. Soc. Exp. Biol. Med. 137:1225-1227.
49. Miller, J. K., J. Hacking, and V. I. Gross. 1973. Stillbirths, neonatal mortality and still litters in pigs associated with ingestion of Fusarium toxin by pregnant sows. Vet. Rec. 93:555-559.
50. Millington, A. J., C. M. Francis, and N. R. McKeown. 1964. Bioassay of annual pasture legumes. II. The oestrogenic activity of nine strains of *Trifolium subterranean* L. Aust. J. Agric. Res. 15:527-536.
51. Mirocha, C. J., and C. M. Christensen. 1974. Oestrogenic mycotoxins synthesized by *Fusarium*. pp. 129-148. In I. F. H. Purchase (ed.). Mycotoxins. Elsevier Sci. Publ. Co., New York, N.Y.
52. Mirocha, C. J., C. M. Christensen, and G. H. Nelson. 1968. Physiologic activity of some fungal estrogens produced by *Fusarium*. Cancer Res. 28:2319-2322.
53. Mirocha, C. J., C. M. Christensen, and G. H. Nelson. 1971. F-2 (zearalenone) estrogen mycotoxin from *Fusarium*. pp. 107-138. In S. Kadis, A. Ciegler, and S. J. Ajl (eds.). Microbial toxins, a comprehensive treatise. Vol. 7, Academic Press, New York, N.Y.
54. Morley, F. H. W., D. Bennett, A. W. H. Braden, K. E. Turnbull, and A. Axelsen. 1968. Comparison of mice, guinea pigs and sheep as test animals for bioassay of oestrogenic pasture legumes. Proc. N. Z. Soc. Anim. Prod. 28:11-21.

55. Naim, M., B. Gestetner, S. Zilkah, Y. Birk, and A. Bondi. 1974. Soybean isoflavones, characterization, determination and antifungal activity. J. Agr. Food Chem. 22:806-810.
56. Nash, A. M., A. C. Eldridge, and W. J. Wolf. 1967. Fractionation and characterization of alcohol extractables associated with soybean proteins. J. Agric. Food. Chem. 15:102-108.
57. Newton, J. E., and J. E. Betts. 1973. The effects of red clover (*Trifolium pratense* var. *redhead*), white cloves (*Trifolium repens* vars. 5100) or perennial ryegrass (*Lolium perenne* var. 523) on the reproductive performance of sheep. J. Agri. Sci., Camb. 80:323-327.
58. Noble, R. L., and L. Hoover. 1975. A classification of transplantable tumors in Nb rats controlled by estrogen from dormancy to autonomy. Cancer Res. 35:2935-2941.
59. Noteboom, W. D., and J. Gorski. 1963. Estrogenic effect of genistein and coumestrol diacetate. Endocrinology 73:736-739.
60. Okey, A. B., and G. P. Bondy. 1978. Δ^9-Tetrahydrocannabinol and 17β-estradiol bind to different macromolecules in estrogen target tissue. Science 200:312-314.
61. Olah, A. F., and R. T. Sherwood. 1971. Flavones, isoflavones and coumestans in alfalfa injected by *Ascochyta imperfecta*. Phytopathology 61:65-69.
62. Olah, A. F., and R. T. Sherwood. 1973. Glycosidase activity and flavonoid accumulation in alfalfa infected by *Ascochyta imperfecta*. Phytopathology 63:739-742.
63. Palmiter, R. D., and E. R. Mulvihill. 1978. Estrogenic activity of the insecticide kepone on the chicken oviduct. Science 201:356-358.
64. Perry, T. W., M. Stob, D. A. Huber, and R. C. Peterson. 1970. Effect of subcutaneous implantation of resorcylic acid lactone on performance of growing and finishing beef cattle. J. Anim. Sci. 31:789-793.
65. Rich, J. R., and N. T. Keen. 1975. Association of coumestans with the hypersensitive resistance of lima bean to *Pratylenchus scribneri*. J. Nematol. 7:328-329.
66. Richardson, T., and N. Mattarella. 1977. Hormonal substances in human milk, cow's milk and dairy products. J. Food Prot. 40: 57-64.
67. Schoental, R. 1974. Role of podophyllotoxin in the bedding and dietary zearalenone on incidence of spontaneous tumors in laboratory animals. Cancer Res. 34:2419-2420.
68. Schoental, R. 1974. Zearalenone in the diet, podophyllotoxin in the wood shavings bedding, are likely to affect the incidence of "spontaneous" tumors among laboratory animals. Brit. J. Cancer 30:181.
69. Sharaf, A., and S. Negm. 1973. Oestrogenic activity of *Zea mays* oil. Qual. Plant Mater. Veg. 22:249-252.
70. Sharp, G. D., and I. A. Dyer. 1972. Zearalanol metabolism in steers. J. Anim. Sci. 4:176-179.
71. Shemesh, M., H. R. Lindner, and N. Ayalon. 1972. Affinity of rabbit uterine oestradiol receptor for phytoestrogens and its use in a competitive protein-binding radioassay for plasma coumestrol. J. Reprod. Fert. 29:1-9.
72. Shipchandler, M. I. 1975. Chemistry of zearalenone and some of its derivatives. Heterocycles. 3:471-520.
73. Shutt, D. A. 1976. The effect of plant oestrogens on animal reproduction. Endeavor 35:110-113.
74. Shutt, D. A., and R. I. Cox. 1972. Steroid and phytoestrogen binding to shape uterine receptors in vitro. J. Endocr. 52: 299-310.
75. Shutt, D. A., R. H. Weston, and J. P. Hogan. 1970. Quantitative aspects of phytoestrogen metabolism in sheep fed on subterranean clover (*Trifolium subterraneum* cultivar *clare*) of red clover (*Trifolium pratense*). Aust. J. Agric. Res. 21:713-722.
76. Solomon, J., M. A. Cocchia, and R. DiMartino. 1977. Effect of delta-9-tetrahydrocannabinol on uterine and vaginal cytology of ovariectomized rats. Science 195:875-877.
77. Spencer, R. R., E. M. Bickoff, R. E. Lundin, and B. E. Knuckles. 1966. Lucernol and sativol, two new coumestans from alfalfa (*Medicago sativa*). J. Agric. Food Chem. 14:162-165.
78. Spencer, R. R., B. E. Knuckles, and E. M. Bickoff. 1966. 7'Hydroxy-11, 12-dimethoxycoumestan. Characterization and synthesis. J. Org. Chem. 31:988-989.
79. Stoloff, L., S. Henry, and O. J. Francis Jr. 1976. Survey for aflatoxins and zearalenone in 1973 crop corn stored on farms and in country elevators. J. Assoc. Off. Anal. Chem. 59:118-121.
80. Tamura, S., C. Chang, A. Suzukki, and S. Kumai. 1969. Chemical studies on clover sickness. 1. Isolation and structural elucidation of two new isoflavonoids in red clover. Agric. Biol. Chem. 33:391-397.
81. Ueno, Y., and K. Kubota. 1976. DNA-attacking ability of carcinogenic mycotoxins in recombination-deficient mutant cells of *Bacillus subtilis*. Cancer Res. 36:445-451.
82. Utian, W. H. 1973. Comparative trial of P1496, a new non-steroidal oestrogen analogue. Brit. Med. J. 1:579-581.
83. Vanyi, A., and E. Szailer. 1974. Investigation of the cytotoxin effect of F-2 (zearalenone) in various monolayer cell cultures. Acta. Vet. Acad. Sci. Hung. 24:407-412.
84. Verdeal, K. 1978. Dietary estrogens and hormone-dependent mammary cancer. M. S. thesis, University of Wisconsin, Madison, Department of Food Science.
85. Wong, E. 1975. The isoflavonoids. pp. 743-800. In J. B. Harborne, T. J. Mabry, and H. Mabry (eds.). The flavonoids. Academic Press, New York, N.Y.
86. Wong, E., and O. S. Flux. 1962. The oestrogenic activity of red clover isoflavones and some of their degradation products. J. Endocrin. 24:341-348.
87. Young, I. J., B. A. Knights, and J. R. Hillman. 1977. Oestradiol and its biosynthesis in *Phaseolus vulgaris* L. Nature 267:429.