of the experiment if some of the embryos of the strain used had been of a different leaf stage.

Some other advantages of the stage-per-day method are not as obvious. Each of the first seven circles in Fig. 1 represents a sample of five plants, and each of the remaining circles represents a sample of 10 plants per harvest. The sample size for the x's, however, ranges from 3 to 29, being a function of the duration of the stage. It follows that, except in cases of linear-growth rates, equal sample size cannot be attained in the mid-point method. Nor is there any possibility of determining somewhat more subtle changes in growth rate within a stage except by classification into morphological substages (*4*). Although the mid-point method requires samples of equal size and sampling at equal time intervals, these conditions do not have to be met in the stage-per-day method, since the data from one sample are not combined with those from the others.

As stated earlier, an advantage of the mid-point method is that it permits mathematical determination of the duration of stage, whereas this must be determined graphically in the stage-per-day method. However, depending on the precision desired, points can be obtained close enough to one another to assure accurate determination in the latter case. Horizontal ticks on the broken line of the graph and the lower bar at the top of Fig. 1 represent duration of plastochrons 7–13 thus ascertained.

References

1. E. C. Abbe and B. O. Phinney, *Am. J. Botany* **38**, 737 (1951).
2. E. C. Abbe and O. L. Stein, *ibid.* **41**, 285 (1954).
3. O. L. Stein, master's thesis, Univ. of Minnesota (1952).
4. O. L. Stein and E. C. Abbe, *Proc. Minn. Acad. Sci.* **17**, 97 (1949).

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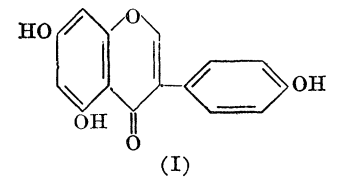
Estrogenic Activity of Some Isoflavone Derivatives*

Edmund Cheng, Lester Yoder, Charles D. Story, Wise Burroughs

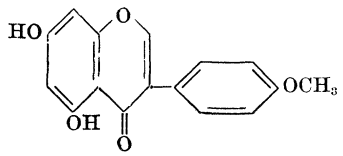
Iowa Agricultural Experiment Station, Ames

Interest in natural estrogenic compounds present in livestock feeds has been stimulated by the beneficial results obtained from the addition of diethylstilbestrol to cattle rations (*1*). The presence of estrogenic substances in subterranean clover interfering with the breeding performance in sheep (*2*) has led to the isolation of an isoflavone derivative, genistein, as one of the active substances (*3*). Cheng *et al.* (*4*) reported that genistein, as well as the glucoside of genistein, known as genistin, was estrogenic as detected by the mouse uterine response procedure. The estrogenic activity of genistein has further been confirmed by Carter *et al.* (*5*).

Since there are several known isoflavone compounds present in natural plant material, it is of interest to determine which of these compounds are estrogenic. Chemical synthesis of four isoflavone derivatives, genistein (I), biochanin A (II), daidzein (III), and formononetin (IV), has been completed in this laboratory (*6*). Their structural formulas are shown here.



(I)



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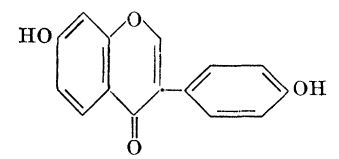
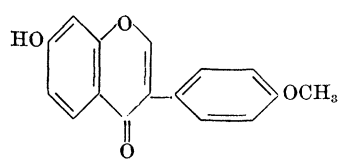


(III)



(IV)

Unpublished data in this laboratory indicated that both synthetic and naturally occurring genistein have equally potent estrogenic activity. Consequently, only synthetic isoflavone compounds were tested in the present experiment. These compounds were fed to mice at a level of 1.25 mg/g of ration in testing estrogenic activity using the uterine response technique (*7*). The respective isoflavone compounds were first dissolved in ethanol, then mixed with the basal ration, and the ethanol was evaporated from the completely mixed ration. Since the mice consumed an average of 2 g of diet daily, the average intake of the respective compounds was 2.5 mg daily over the experimental period of 4 days. The results of this experiment are presented in Table 1.

It is readily apparent that each of these isoflavones is estrogenic in nature. Daidzein appears to be the most active substance. Genistein and biochanin A have approximately equal activity. Formononetin showed

Table 1. Estrogenic activity of some isoflavone derivatives.

No. of mice	Treatment	Avg. uterine weight (mg)	Approx. potency*
6	Normal control	6.4 ± 0.8†	
6	2.5 mg biochanin A	20.9 ± 3.1	0.033
5	2.5 mg daidzein	26.6 ± 4.1	.042
6	2.5 mg formononetin	8.9 ± 1.2	.009
5	2.5 mg genistein	19.3 ± 1.3	.030
6	0.01 μg stilbestrol	9.4 ± 0.8	
6	0.02 μg stilbestrol	15.7 ± 1.5	
6	0.04 μg stilbestrol	22.2 ± 2.1	
5	0.08 μg stilbestrol	46.2 ± 4.7	

* Expressed as micrograms of diethylstilbestrol activity.
† Standard deviation.

the least estrogenic activity. From the structural formula, it can be seen that formononetin is the only compound tested that has only one free hydroxyl group. Since the activity in many synthetic estrogenic compounds is known to be related to the number and arrangement of hydroxyl groups (*8*), it is not surprising that formononetin is less active than the other compounds tested.

Biochanin A was recently isolated from red clover and found to be estrogenic (*9*). The isoflavone daidzein has not been reported as being present in nature. However, its glucoside, daidzein, has been isolated from soybean-oil meal (*10*).

It appears likely that the estrogenic activity in livestock feeds is primarily due to compounds classified chemically as isoflavone derivatives. The estrogenic potency of these isoflavone compounds is low when compared with that of diethylstilbestrol; nevertheless, when one considers the large amount of such feeds as legume hay and soybean-oil meal that are consumed by farm animals, enough estrogenic substance may be present in these feeds to exert an important influence upon their physiological functions. The effect may be adverse if too much of the estrogenic substance is present, as in the case of subterranean clover (*2*). On the other hand, if the correct amount is present, the effect may be as beneficial as diethylstilbestrol in stimulating live-weight gain in beef cattle as shown by Burroughs *et al.* (*1*).

References and Notes

* Journal paper No. J-2530, Iowa Agricultural Experiment Station, Ames, project 1208.
1. W. Burroughs *et al., Science* **120**, 66 (1954).
2. H. W. Bennetts *et al., Australian Vet. J.* **22**, 2 (1946).
3. R. B. Bradbury and D. E. White, *J. Chem. Soc.* **1951**, 3447 (1951).
4. E. Cheng *et al., Science* **118**, 164 (1953).
5. M. W. Carter *et al., Proc. Soc. Exptl. Biol. Med.* **84**, 506 (1953).
6. L. Yoder *et al., Proc. Iowa Acad. Sci.*, in press.
7. E. Cheng *et al., J. Animal Sci.* **12**, 507 (1953).
8. U. V. Solmssen, *Chem. Revs.* **37**, 481 (1945).
9. G. S. Pope *et al., Chemistry & Industry* **1953**, 1092 (1953).
10. E. Walz, *Ann. Chem.* **489**, 118 (1931).

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Antiaccelerator and Antiarrhythmic Cardiac Action of Synthetic Steroid Alkamines

S. Margolin,* Go Lu,† J. Yelnosky, A. Makovsky

Pharmacological Research Department, Schering Corporation, Bloomfield, New Jersey

The demonstration of antiaccelerator cardiac activity for the synthetic steroid secondary amine, 20-(5′methyl-2′-piperidyl)-5-pregnen-3,20-diol (*1, 2*), prompted the synthesis of several steroids with alkamine substitutions in the 16 position by D. Gould and E. B. Hershberg in the Chemical Research Division of Schering Corporation. Our objective was a potent antiaccelerator agent suitable for studies in human beings.

Antiaccelerator cardiac action was first observed in our series with Sch 1837, 16-N-piperidinopregnenolone. This substance and closely related derivatives are strongly convulsant in nonanesthetized dogs at antiaccelerator dosages. However, the activity in this structure encouraged further synthetic efforts, and pharmacological studies brought attention to cyclohexylamine derivatives of pregnenolone. Sch 2331, 16-cyclohexylaminopregnenolone, displayed antiaccelerator activity, and antagonized experimentally induced auricular and ventricular arrhythmias. Sch 2602, 16-cyclohexylaminopregnandiol, was highly effective as an antiaccelerator and antiarrhythmic agent, but it disclosed no important advance over Sch 2331 from a toxicity standpoint. Although these two compounds provided a distinct advance over earlier preparations and could display cardiac activity in nonanesthetized dogs, they induced central stimulation at doses only slightly above those causing antiaccelerator or antiarrhythmic activity. Further experiments demonstrated the greater activity of Sch 2684, 16-cyclohexylamino-allopregnandiol, and indicated its relative safety. Sch 2331, Sch 2602, and Sch 2684 present a new active chemical series with a noteworthy pharmacological combination of highly effective cardiac antiaccelerator and antiarrhythmic action.

The antiaccelerator effect of Sch 1837 and Sch 2331 was suggested by the observation of a distinct slowing of heart rate after injection of these substances into normal and atropinized anesthetized dogs. The compound prepared by Uhle (*1*) and Sch 2684 behave similarly in such tests. Evidence for a direct cardiac action was obtained with isolated perfused rabbit hearts (Langendorff preparation) and by dog heart-lung experiments (after Krayer, *3*). The positive chronotropic action of epinephrine on isolated rabbit hearts was partially or completely blocked by 5 to 50 μg per heart of Sch 2684 without diminishing the positive inotropic response to the epinephrine. In the dog heart-lung preparations, 7 mg of Sch 2684 in a single dose blocked the cardiac acceleration induced by a continuous infusion of epinephrine hydrochloride at a rate of 10 to 45 μg/min, whereas the cardiac output remained essentially unaltered. Marked slowing of the pulse rate in preliminary clinical trials with Sch 2684