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Uterotropic Activity of *cis* and *trans* Isomers of Zearalenone and Zearalenol†

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The *cis* and *trans* isomers of zearalenone [2,4-dihydroxy-6-(10-hydroxy-6-oxo-1-undecenyl)-benzoic acid μ-lactone] and zearalenol [2,4-dihydroxy-6-(6,10-dihydroxy-1-undecenyl)-benzoic acid μ-lactone] were tested for uterotropic activity in the white rat. The metabolites were administered through the oral route (per os) and by topical application to the freshly shaven skin on the back. *cis*-Zearalenone was significantly more active than *trans* when fed orally to the rats in the diet or when applied topically by skin application. However, the *cis* isomer of zearalenol was not significantly different than its *trans* isomer. *trans*-Zearalenone was less active than *trans*-zearalenol.

Zearalenone is an estrogenic metabolite produced by various species of *Fusarium* colonizing maize, oats, barley, wheat, and sorghum. This fact became of economic importance because of the discovery that zearalenone was the causal factor of hyperestrogenism and infertility in swine (1, 4).

Peters (2) reported that the *trans*- 1',2 double bond in naturally occurring zearalenone (Fig. 1I) can be isomerized photochemically to the *cis* form (Fig. 1III) and that the *cis* isomer has slightly less uterotropic activity in mice than the *trans* isomer. Peters (2) also tested both diastereomers of *cis*- and *trans*-zearalenol (Fig. 1II and IV) and found that both *cis* isomers (high and low melting point) had significantly more uterotropic activity than their respective *trans* isomers.

Wolf and Mirocha (4) examined the structure-activity relationships of the *cis* and *trans* isomers of zearalenone in regulating the sexual stage of *Fusarium roseum* and found no difference in activity in the fungus system. During this time, it was decided to reexamine the uterotropic activity of these isomers in rats.

cis-Zearalenone [2,4-dihydroxy-6-(10-hydroxy-6-oxo-1-undecenyl)-benzoic acid μ-lactone] was prepared by irradiation of zearalenone with ultraviolet light according to the procedures of Peters (2), which yielded the *cis* isomer within 30 h (mp 137.0 to 137.5°C).

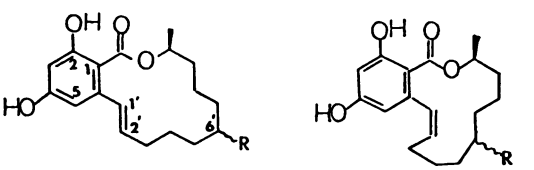
cis-Zearalenol [2,4-dihydroxy-6-(6,10-dihydroxy-1-undecenyl)-benzoic acid μ-lactone] was prepared by reduction of the purified *cis*-zearalenone with sodium borohydride in ethanol. The diastereomeric mixture of zearalenol was purified on Silica Gel G by thin-layer chromatography (chloroform-ethanol, 93:3). No attempt was made to separate each of the diastereomers.

The *cis*-isomers of both zearalenone and zearalenol are stable and retain their structural integrity in biological systems. Thus, *cis*-zearalenone either placed in actively metabolizing *Fusarium* cultures or fed to rats was recovered from the system (either by cultures or in urine) after 24 h solely as *cis*-zearalenone.

Uterotropic activity. Both isomers were tested on 20-day-old, white weanling female rats using two methods of administration: (i) incorporation into balanced ration (per os) for one feeding or (ii) single topical application on the shaved skin.

The rats were kept on experiment for 3 days after treatment, after which they were sacrificed and the uterus was excised and weighed fresh. Three replications were used in each dose treatment.

As shown in Table 1, (i) *cis*-zearalenone was more active than the *trans* isomer in both the



		R
I	trans-zearalenone	=O
II	trans-zearalenol	-OH
III	cis-zearalenone	=O
IV	cis-zearalenol	-OH

FIG. 1. *Structures of trans- (left) and cis- (right) zearalenone and -zearalenol.*

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TABLE 1. *Uterotropic activity of cis and trans isomers of zearalenone and zearalenol (diastereomeric mixture) in rats*[a]

Dose (mg)	Zearalenone				Zearalenol			
	Feeding test[b]		Skin test[c]		Skin test I[c]		Skin test II[c]	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
0.2	60[d]	40					89	114
0.25			134[d]	58	191	168		
0.40	109	71					152	163
0.50			232[e]	46				
0.75					182	214		
0.80	236	164					154	139
1.25	266[e]	115	307[f]	84	266[d]	112		
Average	168[f]	98	224[e]	63	213	165	132	139

[a] Weight of the rat uteri is expressed in milligrams of fresh weight. The weight of the uteri of control rats ranged between 27 and 40 mg. Each mean is the average value of three rats.
[b] Isomers were fed by incorporating into the rat's diet.
[c] Isomers were applied topically on the shaved skin of the back of the rat.
[d] *cis* isomer was significantly different from *trans* isomer at the 10% level by Student's *t* test: $P < 0.1$.
[e] *cis* isomer was significantly different from *trans* isomer at the 1% level by Student's *t* test: $P < 0.01$.
[f] *cis* isomer was significantly different from *trans* isomer at the 5% level by Student's *t* test: $P < 0.05$.

TABLE 2. *Uterotropic activity of trans-zearalenone versus trans-zearalenol (diastereomeric mixture) in rats*[a]

Dose (mg)	Expt I		Expt II	
	trans-Zearalenone	*trans*-Zearalenol	*trans*-Zearalenone	*trans*-Zearalenol
0.2	43.3 ± 16.3[b]	113.7 ± 37.4		
0.25			76.7 ± 60.5[c]	168.3 ± 31.5
0.40	31.0 ± 2.6[d]	162.7 ± 9.6		
0.75			42.7 ± 19.4[b]	213.7 ± 75.5
0.80	39.7 ± 9.5[d]	138.67 ± 29.7		
1.25			81.3 ± 24.0	112.3 ± 21.3

[a] Expressed as milligrams of fresh weight of rat uteri. Both test materials were applied topically to the skin.
[b] $P < 0.05$.
[c] $P < 0.1$.
[d] $P < 0.01$.

oral and skin application tests; (ii) both isomers showed a dose-response relationship; and (iii) the skin test showed less variation than the oral feeding test. The *cis* isomer of zearalenol, however, was not significantly different from its *trans* isomer. *trans*-Zearalenone was less active than *trans*-zearalenol (Table 2).

The enhanced activity of *cis*-zearalenone agrees with the fact that the *cis* isomer has a higher binding affinity to the specific estrogen receptor protein of the rat than does its *trans* isomer (D. T. Kiang, B. J. Kennedy, S. V. Pathre, and C. J. Mirocha, Proc. Natl. Acad. Sci. U.S.A., in press).

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