Uterotropic and Anti-Implantation Activities of Certain Resorcylic Acid Lactone Derivatives (35521)

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There are many synthetic compounds, possessing a wide variety of chemical structures, which demonstrate estrogenic activity. The numerous reports of genital abnormalities in swine fed on moldy corn prompted Stob *et al.* (1) to investigate the microorganisms implicated in the observed syndrome. One of the predominant microorganisms identified in their culture samples of spoiled-corn molds was the fungus, *Gibberella zeae* (*Fusarium graminearum*). From extracts of this fungus, Stob and his co-workers isolated and partially characterized a metabolite which induced marked uterotropic responses in a variety of laboratory animals. These studies were fully confirmed by Christensen *et al.* (2) and Mirocha *et al.* (3, 4). Using mass spectrometric and NMR methods, Urry *et al.* (5) were able to show that the estrogenic substance contained olefinic, ketonic, phenolic hydroxyl, and ester groups in a β-resorcylate structure as shown in Fig. 1.

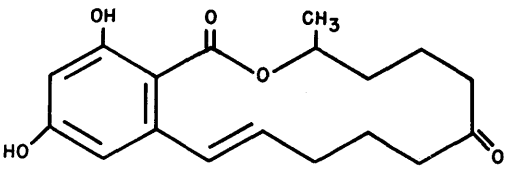


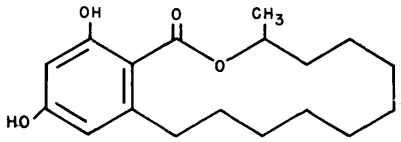
Figure 1

The metabolite was given the trivial name "zearalenone" to designate its being a resorcylic acid lactone (RAL) derivative produced by *Gibberella zeae*. Subsequently, Urry and his associates described the chemical preparation of a number of other RAL's.

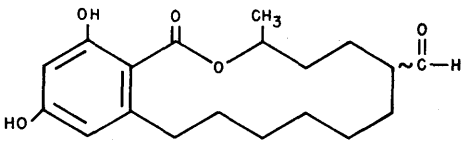
In the course of evaluating the biologic spectra of these substances it was noted that certain modifications of one, zearalane,[1] brought about a very considerable enhancement of uterotropic activity. This report is concerned primarily with the oral estrogenic activity of zearalane and its derivatives. The criteria used to determine such activity were uterine weight increase in immature rats and antifertility potency in mature rats.

Materials and Methods. The chemical structures of zearalane (Compound I) and its analogues used in these studies are shown in Figure 2. Compound II is composed of Isomers A and B. Compound III consists of Isomers C and D.

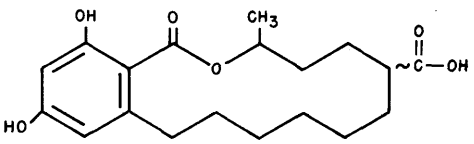
Uterotropic assays were conducted using



ZEARALANE
(COMPOUND I)



7'-FORMYL ZEARALANE (COMPOUND II)
THIS IS A MIXTURE OF ISOMERS A AND B



7'-CARBOXY ZEARALANE (COMPOUND III)
THIS CONSISTS OF ISOMERS C AND D

Figure 2

[1] The zearalane used in the studies described in this manuscript was supplied by the Commercial Solvents Corporation. The zearalane analogues were prepared by Dr. N. P. Jensen and Miss Susan Schmitt of the Department of Chemical Research in the Merck Sharp & Dohme Research Laboratories.

TABLE I. Oral Estrogenicity of Zearalane and Its 7′-Formyl and 7′-Carboxy Derivatives in the Rat.

Treatment	No. of animals	Total dose (μg)	Mean[a] uterine wt (mg)	Estrogenic potency relative to DES (95% confidence limits)
Control	23	—	28	—
DES	22	0.1	39	—
	24	0.3	54	—
	18	0.9	80	—
Compound I (zearalane)	6	100	39	
	6	300	50	0.00016 (0.0001–0.00026)
	6	900	69	
	6	2700	89	
Compound II (7′-formyl zearalane)	6	10	47	
	6	20	63	0.025 (0.019–0.034)
	6	40	84	
Isomer A of 7′-formyl zearalane	6	20	41	
	6	60	49	0.009 (0.007–0.012)
	6	180	80	
Isomer B of 7′-formyl zearalane	6	5	45	
	6	10	60	0.047 (0.035–0.065)
	6	20	89	
Compound III (7′-carboxy zearalane)	6	5	50	
	6	10	61	0.050 (0.037–0.068)
	6	20	79	
Isomer C of 7′-carboxy zearalane	6	2.5	47	
	6	5	70	0.096 (0.072–0.132)
	6	10	74	
	5	20	97	
Isomer D of 7′-carboxy zearalane	6	6.67	49	
	6	20	62	0.009 (0.005–0.015)
	6	60	73	

[a] The uterine weight values for the control and DES-treated groups are averages compiled from several assays.

21-day old female Carworth rats. Six rats were used for each dose level, and the doses were given by gavage in 0.2 ml of sesame oil daily for 3 days. The animals were sacrificed the day after the final treatment and uteri were removed, blotted, and weighed. The uterotropic response was measured against the reference compound, diethylstilbestrol (DES).

For the determination of antifertility activity, adult virgin female (Holtzman) rats were mated with fertile males, and vaginal smears were checked daily for the presence of sperm. The day sperm were detected was considered day 1 of pregnancy. Test compounds were administered in 0.2 ml of sesame oil as a single oral dose on day 2. In our animals, day 2 of pregnancy has been shown to be particularly susceptible to the action of "estrogens." The animals were autopsied on day 9 and the number of implantations was recorded. The effective dose (MED_{100}) was the smallest amount of compound necessary to inhibit implantation completely in all animals.

Results. Table I summarizes the results of several oral uterotropic assays in which zearalane (Compound I) and its 7′-formyl and 7′-carboxy derivatives were compared to DES. The 7′-formyl derivatives included

Compound II and its Isomers, A and B. The 7′-carboxy derivatives were Compound III and its two components, Isomers C and D. Wide differences in uterotropic activity were noted among the various compounds. Zearalane (Compound I) was the least potent, having less than one-tenth the activity of any of the derivatives bearing a 7′-formyl or 7′-carboxy group. In comparison with DES, zearalane was only about 0.0001–0.0002 times as active.

Most of the activity of Compound II (7′-formyl zearalane) was centered in the Isomer B component. That substance was about five times as active as Isomer A and twice as potent as Compound II itself. Uterotropic potencies relative to DES were 0.025, 0.009, and 0.047 for Compound II, Isomer A, and Isomer B, respectively.

Among the 7′-carboxy zearalanes, Isomer C was the most active. This substance was found to have about ten times the uterotropic activity of Isomer D and about twice that of Compound III. With reference to DES, the potencies of Compound III, Isomer C and Isomer D were 0.05, 0.096, and 0.009, respectively.

Anti-implantation activity was noted with zearalane and all its derivatives. The dosages at which 100% effectiveness was obtained for each compound are shown in Table II. With the addition of a formyl group in the 7′-position, antifertility properties increased substantially, most of the activity being in the Isomer B component. Substitution of the 7′-carboxy group resulted in a very marked increase in activity. The mixture (Compound III) and its isomers all demonstrated activity essentially within the same range, although Isomer C was slightly more potent.

Discussion. The addition of formyl or carboxy groups at the 7′-position markedly enhances the estrogenicity of the RAL derivative, zearalane. In Isomer C the estrogenicity of zearalane was increased more than 100-fold by addition of the 7′-carboxy group. Isomer B, which contains a 7′-formyl group, is more than 40 times as estrogenic as zearalane. In comparison with DES, Isomer B has about one-twentieth the estrogenic activity while Isomer C has one-tenth the potency.

TABLE II. Anti-implantation Activity of Zearalane and Its 7′-Formyl and 7′-Carboxy Derivatives in the Rat.

Compound	Anti-implantation activity MED_{100} (mg)
DES	0.015
I (zearalane)	14.0
II (7′-formyl zearalane)	5.0
Isomer A of 7′-formyl zearalane	10.0
Isomer B of 7′-formyl zearalane	2.5
III (7′-carboxy zearalane)	0.5
Isomer C of 7′-carboxy zearalane	0.1
Isomer D of 7′-carboxy zearalane	0.4

Since aldehydes readily undergo oxidation to the corresponding acids in the body, it is very likely that the 7′-formyl isomers were converted after administration and exerted their biological effects as acids, rather than as aldehydes. Thus, it is of particular interest that the more active of the carboxy isomers, Isomer C, was derived chemically from the more active formyl isomer, B, by oxidation with Jones' reagent (N. P. Jensen, manuscript in preparation). The less active formyl Isomer A was similarly oxidized to the weak carboxy Isomer D.

No attempt was made to determine how the compounds prevented ovum implantation, but it may be assumed that passage through the oviducts was accelerated. This is known to occur when estrogens are administered to the rat (6). Ova arrive in an asynchronous uterus (7) and are unable to implant.

A primary concern in these studies was whether a separation of uterotropic and anti-implantation activities would be revealed. It was evident from data summarized in Table I and II that prevention of implantation was, in general, positively correlated with estrogenicity. However, there was also considerable variation between compounds in the ratio of estrogenic to anti-implantation activity. At least part of that discrepancy may be explained by the fact that estimates of estrogenicity based on uterine weight assays are relatively imprecise (8). With this possible shortcoming in mind, it was concluded that

an unequivocal separation of activities had not been observed.

Summary. Uterotropic and antifertility properties of the resorcylic acid lactone derivative, zearalane, and its 7′-formyl and 7′-carboxy analogues have been evaluated. Additions at the 7′-position enhanced the estrogenic and anti-implantation activities of zearalane. The more active isomer of 7′-carboxy zearalane proved to be at least 100 times as estrogenic as zearalane and one-tenth as active as DES. Anti-implantation potency was increased in a corresponding manner.

There was no unequivocal separation of estrogenic and anti-implantation activities noted in any of the compounds tested.

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