ZERANOL

IDENTITY

Chemical name:	(3S, 7R)-3, 4, 5, 6, 7, 8, 9, 10, 11, 12-decahydro-7,14,16-trihydroxy-3-methyl-1H -2-benzoxacyclotetradecin-1-one 6-(6, 10-dihydroxyundecyl)-β-resorcylic acid α-lactone
Synonyms:	α-zearalanol P-1496
Structural formula:	Structural formula of zeranol and related resorcylic acid lactones (RAL)compounds are given in Figure 1.
Molecular formula:	$C_{18}H_{26}O_5$
Molecular weight:	322.40

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance:	White, crystalline, odourless powder
Melting point:	181-185°C
Optical rotation:	$[\alpha]_D^{25}$ = + 44.5° - 47.5° (c = 1.0 in methanol)
UV_{max}:	218, 265, 304 nm

(Windholz, 1983)

Technical active ingredient:

Zeranol is obtained from the mycotoxin zearalenone which is produced in submerged culture of the fungus Fusarium graminearum (Gibberella Zeae). Zearalenone is modified by Raney nickel reduction of the 7-ketone to a mixture of α- and β- hydroxylated compounds. The concomitant hydrogenation of the unsaturation of C-11, 12 yields a mixture of α- and β-zearalanol. The commercial formulation of zeranol contains specifically the α diastereomer.

Total impurities:	$\leq$ 2 g/100 g	
Nature of impurities:	Taleranol (β-zearalanol):	$\leq$ 1.5%
	Zearalanone:	$\leq$ 0.15%
	Zearalenone:	$\leq$ 0.01%

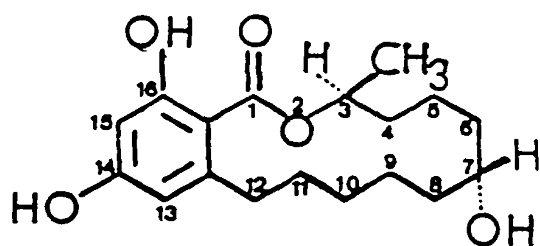
Natural occurrence:

Zeranol has been demonstrated to be a naturally produced metabolite of seven zearalenone producing isolates of Fusarium spp. The seven strains of

Fusaria (six different species) were isolated from finished feeds or forage plants (clover or alfalfa). This direct natural production of zeranol (and taleranol) by Fusarium isolates is important because it suggests the occurrence of these derivatives in animal feedstuffs. Practically, this means any tissue residues of zeranol, its metabolites and other resorcylic acid lactones found in slaughter cattle may be of natural origin and not necessarily from anabolic implant treatment. (Richardson, et al., 1985)

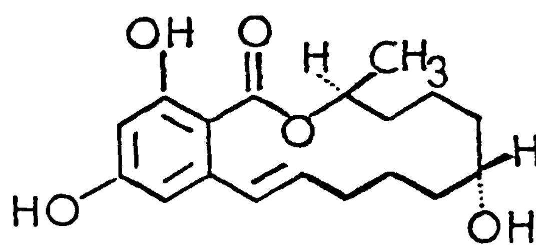
FIGURE I

RAL Nomenclature



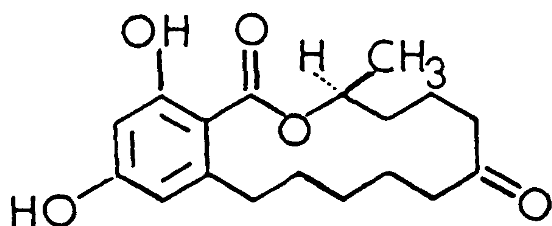
zeranol
α-zearalanol

P-1496
HMTHFES

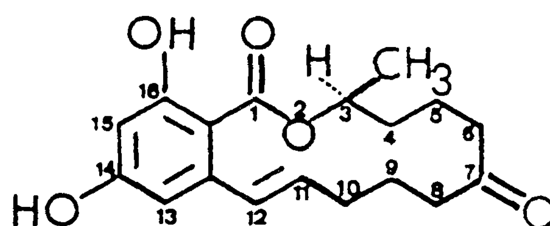


α-zearalenol

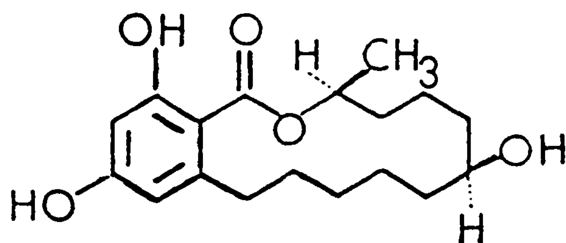
P-1504



zearalanone
P-1502

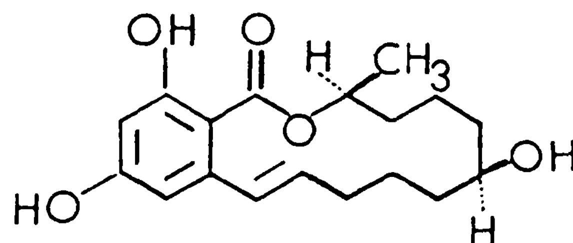


zearalenone
P-1492
FES
F-2 toxin
(The natural "mycotoxin" of the literature)



taleranol
β-zearalanol

P-1560
LMTHFES



β-zearalenol

P-1503

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Zeranol is a non-steroidal anabolic agent. Administration is by subcutaneous implant in the ear of suckling, weaned, growing and finishing cattle. The implant is used alone or with another hormonally active ingredient to increase weight gain and improve feed efficiency.

Dosages:

Ralgro (36 mg zeranol)
Forplix (140 mg trenbolone acetate + 36 mg zeranol)

RADIOLABELED RESIDUE STUDIES

General

The metabolism of zeranol has been studied in various test and target species using (11, 12-^{3}H) zeranol. The radiolabeled purity exceeded 97%. The exchange of ^{3}H was demonstrated to be minimal by injecting a rat subcutaneously (sc) and examining the tissues and excreta for ^3H_2O. The lack of exchange is further substantiated by metabolism studies that demonstrate the stability of the lactone ring in the zeranol molecule.

Table I. Total Residues and their Composition in the Rat

	Liver	Urine[1]	Feces[2]	Blood
Total Residues	84-207 µg/kg	7-13 mg/kg	0.1-0.3 mg/kg	10-17 µg/kg
Female				
Zeranol	25-30%	21%	20%	
Zearalanone	25-30%	26%	50%	
Taleranol	6- 7%	4%	<10%	
Polar[3]	6- 7%	34%	<10%	
Male				
Zeranol	13%	3- 9%		
Zearalanone	20%	"		
Taleranol	13%	"		
Polar	13%	69%		

[1] Characterization was on unhydrolyzed urine
[2] Results were the same for both species
[3] Polar components are defined as those compounds eluting early on the reverse-phase HPLC column.

Rats

Four rats, two male and two female, were orally dosed with 1.5 mg of ^{3}H-zeranol. The amounts of radioactivity in whole blood, liver, urine, and feces were quantitated, then the identities of the ^{3}H-residues in liver, urine and feces were determined using HPLC. The results of the study are summarized in Table I. The major residues in the liver of female rats were zeranol and zearalanone, each comprising 25-30% of the total ^{3}H-residue; taleranol and polar materials each comprised 6-7%. The metabolite zearalanone was the major (20%) residue in the liver of male rats. The major residue in male and female urine was polar material. A reduction in polar material after enzyme hydrolysis with Glusulase occurred with both female and male urine. In feces, zearalanone comprised about 50% of the total ^{3}H-residue and zeranol comprised about 20%. No major sex-related differences were evident. (Mulkey, 1985a)

Cynomolgus Monkeys

Four cynomolgus monkeys, two male and two female, were dosed orally with 1.5 mg of ^{3}H-zeranol. The amounts of radioactivity in serum, liver, urine and feces were quantitated, then the identities of the ^{3}H-residues in liver, urine and feces were determined using HPLC. The results of the studies are summarized in Table II. No sex-related differences were evident. The major residue in liver and feces was zeranol. All of the residue in unhydrolyzed urine was polar material. After hydrolysis with Glusulase (β-glucuronidase/sulfatase), the major radiolabeled residue was zeranol, comprising about 25% of the total ^{3}H-residue. (Mulkey, 1985b)

Table II. Total Residues and their Composition in Cynomolgus Monkeys

	Liver	Urine[1]	Feces	Serum
Total Residues	102-195 μg/kg	5 mg/kg	5 mg/kg	28-49 μg/kg
Composition[2]				
Zeranol	25%	25%	50%	-
Zearalanone	10	5	20	-
Taleranol	10	5	<10	-
Polar	10	5	<10	-

[1] Hydrolyzed
[2] No sex related differences were observed

Cattle

Eighteen beef cattle, nine male and nine female, weighing an average of 221 kg were implanted with 30 mg of ^{3}H-zeranol and slaughtered at various times after implantation: 2, 5, 15, 30, 45 and 65 days. At each time either two males/one female or one male/two females were sacrificed. Two cattle were used as controls. The total residue results of this study are summarized in Table III.

Table III. Total Residues of ^{3}H-Zeranol in Cattle (µg/kg)

Withholding period (days)	Tissue Liver	Kidney	Muscle	Fat[1]	Bile
2	2.5[2]	0.74	0.099	0.10	80
5	8.2	1.7	0.13	0.30	270
15	7.3	1.3	0.10	0.25	230
30	4.2	0.97	0.054	0.26	140
45	3.4	0.89	0.047	0.14	120
65	1.5	0.75	0.044	0.098	56
Detection level	0.07	0.07	0.014	0.035	

[1] Perirenal fat
[2] Each value represents the average of three animals

Tissue residues resulting from ear implantation peaked at 5-15 days and slowly decreased as the implantation time increased. At 65-days, approximately 60% of the initial dose remained at the implant site. Of the 40% depleting from the implant, 12-18% and 21-34% were recovered in the urine and feces, respectively. The residues in urine, feces, liver and kidney were characterized by HPLC. These results are summarized in Table IV.

Table IV. Composition of Residues in Excreta/Tissues

	Urine	Feces[2]	Liver[3]	Kidney
Without Hydrolysis				
Zeranol	-	25%	12%	
Zearalanone	-	10-15%	17%	
Taleranol	-	25%	12%	
Polar	98%	-	52%	
With Hydrolysis[1]				
Zeranol	17%		21.5-32.9%	17.8%
Zearalanone	3.8%		7.1-19.5%	12.6%
Taleranol	43.5%		24.1-32.4%	32.8%
Polar	4%		4.1-20.3%	34.0%

[1] Incubation with Glusulase
[2] 82% of radioactivity extracted
[3] >95% of radioactivity was extracted

The residue levels in edible tissues at all times post-implantation are very low. The maximum residue occurs in liver and never exceeds 10 µg/kg. The residue level in muscle does not exceed 0.13 µg/kg at any time post-implantation.(Tarr, et al., 1984)

Metabolism and Comparative Metabolism

The *in vivo* biotransformation metabolites of ^{3}H-zeranol in tissues, body fluids and excreta were identified and quantified following subcutaneous implantation in the ear of cattle and following oral administration to monkeys and rats. The *in vivo* biotransformation metabolites of ^{3}H-zeranol in body fluids and excreta were identified and quantitated following oral administration to rats, rabbits, dogs, monkeys and man.

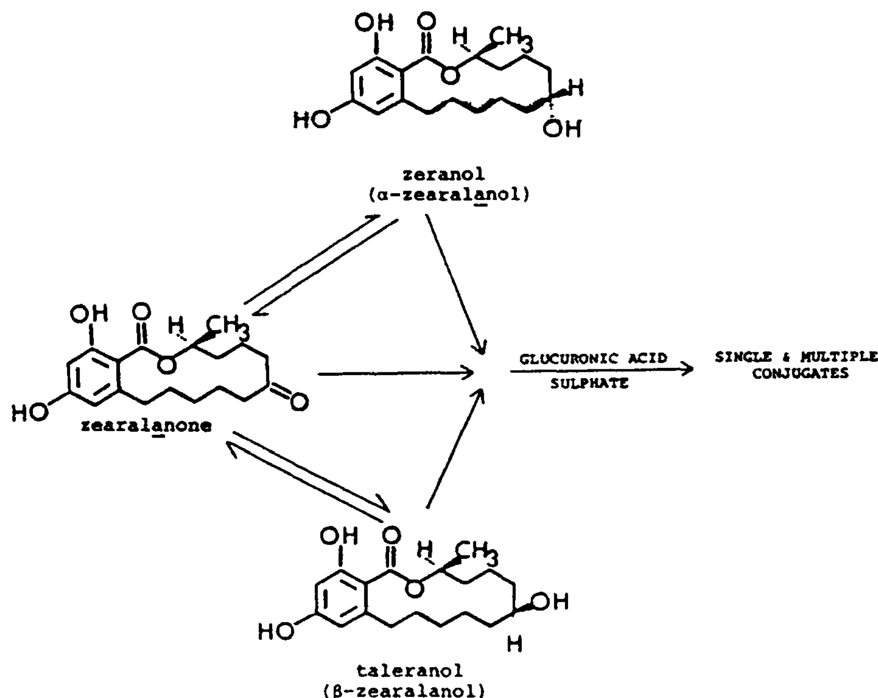
In all mammals studied, zearalanone and taleranol are the only major phase I metabolites of zeranol and all studied mammals metabolize zeranol to both zeralanone and taleranol. The ratios of zeranol to zearalanone to taleranol in tissues and excreta varies from species to species. Zeranol and its metabolites are excreted both as free compounds and as conjugates (glucuronide and/or sulphate).

Additional minor metabolites of unknown identity which demonstrate highly polar characteristics were observed in urine, liver, and feces from ^{3}H-zeranol treated cattle, rats and monkeys. The quantity of these compounds is reduced but not eliminated by prolonged incubation with a crude β-glucuronidase and sulphatase enzyme preparation. It was speculated that these compounds may be multiple conjugates of zeranol and its metabolites. See Figure 2.

The edible tissues of cattle implanted with zeranol contain the same metabolites which laboratory animals and man produce following ingestion of zeranol. Therefore, zeranol safety studies conducted in laboratory animals are directly applicable to human safety evaluations for zeranol and for the auto exposed metabolites. (Tarr, et al., 1984) (Mulkey, 1985a) (Mulkey, 1985b) (Migdalof, et al., 1983)

FIGURE 2

PROPOSED METABOLISM SCHEME FOR ZERANOL IN MAMMALS



RESIDUE STUDIES

Four cows were implanted with Ralgro (36 mg) and slaughtered 70 days after implanting. The tissues were assayed by a radioimmunoassay using a monoclonal antibody. The limits of decision (determination) were reported as 0.278, 0.121, 0.373 and 0.110 µg/kg for muscle, fat, liver and kidney, respectively. The four cows had average values of 0.127, 0.184, 0.299 and 0.157 µg/kg in muscles, fat, liver and kidney, respectively. (Dixon and Russell, 1986)

Twenty-seven steers were implanted with Ralgro (36 mg), biopsied for liver, muscle and fat at 7, 14, 21, 30 or 50 days and slaughtered at 70, 90, or 120 days. The values (ng/kg) at 70 days withholding (where at least 17 animals appeared to be slaughtered) for liver, kidney, muscle, and fat are 200 ± 147, 126 ± 94, 725 ± 886 and 73 ± 40, respectively. The bile at 70 days withholding was reported as 3.28 ± 1.74 mg/l. (Dixon, et al., 1986)

A 13-week-old male veal calf was implanted with 36 mg zeranol and 140 mg trenbolone acetate. A chemiluminescent immunoassay was used to quantitate zeranol, zearalanone and toleranol in urine. A reverse phase HPLC system was used to fractionate the urine extract. Taleranol was the major metabolite in calf urine. (Jansen, et al., 1986)

Six groups of three animals each received one, two, three, four, five and six doses of Ralgro, containing 36 mg/dose. Each dose for animals receiving multiple doses was separated by 65 days, the first dose being given at 60 days of age. All animals were sacrificed 65 days after the last implant. Using a radioimmunoassay, no residues (D.L. = 0.5 µg/kg) were found in muscle, kidney and fat of any of the six groups of calves. No residues (D.L. = 0.5 µg/kg) were found in liver of calves receiving up to three doses. Liver samples of calves receiving four, five and six doses contained mean levels of 0.73, 1.52, and 1.10 µg/kg, respectively. (IMC, undated)

Six steers were implanted with 300 mg trenbolone acetate + 36 mg zeranol and five steers were implanted with 36 mg zeranol. Residues levels of zeranol were obtained by using the authors extraction procedure and the radioimmunoassay of Dixon and Russell (1983) on samples taken at slaughter (67 days after implantation). Only liver (0.349 µg/kg) and kidney (0.076 µg/kg) had residues levels that were significantly over control levels. (O'Keefe, 1984)

Three cows and one young bull were treated with trenbolone acetate (200 mg) + zeranol (36 mg). The cows were implanted at 84 and 56 days before slaughter and the bull was implanted 271 and 183 days before slaughtering. Residues were less than 0.2 µg/kg in muscle and fat, less than 0.3 µg/kg in kidney and less than 0.5 µg/kg in liver. (Gaspar, et al. 1985)

Abusive Conditions of Use

A residue study was conducted jointly by the United States Department of Agriculture and Texas A & M University. Steers were implanted with 24 to 168 mg of zeranol and slaughtered on the fifth day after implanting. A second group of steers were injected with a zeranol/DMSO/saline solution intravenously twice a day for 3 consecutive days. The total i.v. dose administered to these steers ranged from 552 mg to 4128 mg of zeranol. The i.v. injected steers were slaughtered for tissue collection on the third day after the last injection. The results are summarized in Table V. (Cross and Byers, 1987)

Table V. Residue Levels of Zeranol and its Metabolites by Implant and I.V. Dosing

Dose		Muscle (µg/kg)		Liver (µg/kg)	
	Zeranol	Zearalanone	Taleranol	Zeranol	Taleranol
Implant 24 mg	.13	.05	<.02	1.0	-
" 48 mg	.21	.1	<.02	NA	-
" 72 mg	.16	.2	<.02	NA	-
" 120 mg	.16	.09	<.02	NA	-
" 168 mg	.13	.09	<.02	2.9	-
i.v. 552 mg	.14	-	.03	15.0	5.0
" 1374 mg	.29	.19	.06	65.0	40.0
" 2748 mg	.32	.23	.10	50.0	25.0
" 4128 mg	.55	.09	.08	60.0	70.0

NA = not analysed

METHODS OF RESIDUE ANALYSES

General

The radiolabeled study in steers indicates that the total residues under approved conditions of use will not exceed 10 and 2 µg/kg in liver and muscle, respectively. As the residue levels of the parent drug do not exceed 25% of the total residue in either tissue, the level of zeranol should not exceed 2.5 and 0.5 µg/kg in liver and muscle, respectively. Also, the residue levels of parent zeranol have been demonstrated to increase by less than a factor of two under conditions of misuse, such as multiple implants at one time or over a period of time. For these reasons, only those methods with limits of determination of ≤10 and ≤2 µg/kg in liver and muscle, respectively, are discussed.

RIA

Hazleton Raltech, Inc. developed on RIA method for zeranol in animal tissue for International Minerals and Chemical Corporation. The zeranol is extracted from lyophilized tissue using an organic solvent (methanol). Purification of the extracted zeranol is accomplished by solvent partitioning, column chromatography, and finally by high performance liquid chromatography (HPLC). Quantification is done by using antibodies highly specific for zeranol. The method can quantitate zeranol in liver, muscle, kidney and fat at 0.5 µg/kg. This method is cumbersome, requiring two weeks of laboratory work to obtain the results. (IMC, undated)

Several RIA methods have been published using either polyclonal or monoclonal antibodies. These methods are suitable for the monitoring of zeranol in animal tissues in the sub-µg/kg range; however, these methods require the necessity of working with radioactivity and of procurring the radiolabeled standards. Recent method employing chemiluminescent and enzyme detection alleviate these problems. (Jansen, et al., 1986)

CAPILLARY GC

A method progressing from a diethyl-ether extraction of a liver enzyme hydrolysate, and followed by solvent partitioning and capillary gas chromatographic quantitation of the trimethylsilylether (TMS) derivative of zeranol has been developed. Confirmation of the zeranol-TMS derivative utilizes capillary gas chromatography and ion trap detection. The method is linear over

the range of 1 to 10 µg/kg, has a precision of ± 15% and a recovery through the method of 90%. (Boyll, et al., 1987)

GC/MS

The method recovers free and glucuronide conjugates of taleranol, zearalenone, α- and β-zearalenol as well as zeranol. Extraction is by a three phase solvent followed by clean-up on a solid phase cartridge containing a polystyrene divinylbenzene co-polymer resin linked to a quaternary amine anion exchange moiety. Screening is by HPLC with electrochemical detection; quantitation and confirmation is by GC/MS using an on-column derivatization technique and internal standards.

The advantages of this method are the high recoveries attained in the extraction procedure (60 - 80% at the 100 ng/kg level), the small sample size required (5g), and the possibility of automation using commercially available equipment. The main disadvantage of the method is the cost of the equipment; however, this is partially overcome with the high sample through-put attainable. The method is currently being collaboratively studied. A final limit of determination of 0.5 to 1.0 µg/kg is likely. (USDA, 1986)

APPRAISAL

The use of zeranol as an implant in the base of the ear of cattle at a level of 36 mg per animal produces very low levels of drug-related residues in edible tissues at any time post-implantation. The residue levels peak in approximately five days and then slowly decrease to 65-days post-implantation. Presumably, this is due to the encapsulation of the implant with time. The primary constituents of the residues are zeranol, zearalanone and taleranol. The ratio of these constituents to the total residue is relatively constant at post-implantation times up to 65 days, the last withholding time examined. The target tissue is liver, and bile contains the highest level of residue at all times post-implantation. The total residues in liver, kidney, muscle and fat do not exceed 10, 2, .2 and .3 µg/kg, respectively, at any time post-implantation.

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