of illness as a result of ingestion of the virus. In the absence of viremia, all 3 excreted virus in the stools. Homologous neutralizing antibodies developed in the sera of each person. Virus passaged through the human intestinal tract maintained the very low pathogenicity for monkeys when injected intracerebrally.

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Chronic Toxicity Studies on Conidendrins and Conidendrols. (21063)

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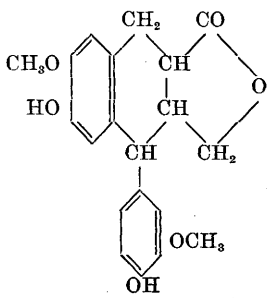
The importance of efficient, non-toxic antioxidants in foods subject to oxidative deterioration is unquestioned. Recently, it has been suggested(1-3) that α- and β-conidendrols could be used as effective antioxidants in edible fats, provided they cause no toxic reactions. This paper reports toxicity studies on these conidendrols, and on α- and β-conidendrin. α-Conidendrin is a partially methylated polyphenol occurring in a number of coniferous trees. (For a description of its occurrence, see Brauns(4)). It can be obtained from the sulfite liquor of the wood-pulp industry. Its chemical structure is:



By inversion at the carbon adjacent to the carbonyl group of the lactone ring, an isomer, β-conidendrin, is formed. Both of these compounds are mild antioxidants. α- and β-Conidendrol (also called α- and β-nonconidendrin) likewise are isomers, and are obtained by demethylation of α-conidendrin(5). They are stronger antioxidants than the conidendrins. Both conidendrols inhibit the rate of peroxide formation in fat when incorporated in the fat in concentrations of 0.01 to 0.1%. The β-isomer is the more effective as a fat antioxidant(3).

Reports on toxicity of these compounds seem to be limited to a short statement(6) on subacute toxicity following intraperitoneal administration to mice. Such administration for several days showed the maximum tolerated daily dose of α- and β-conidendrin to be 500 and 250 mg/kg, respectively, and of α- and β-conidendrol, 62.5 and 113.5 mg/kg, respectively. No statement has been found concerning the metabolism of these compounds in the mammal. However, there are reports that degradation of α-conidendrin can be accomplished by microorganisms(7), although identification of the metabolites has not yet been announced, and that a polyphenol oxidase of microbial origin can degrade conidendrin(8).

Experimental. The effect of continued feeding of diets containing these 4 compounds was studied on weanling albino rats.* The

* The conidendrins and conidendrols were kindly supplied by Drs. W. W. Moyer and W. M. Hearon, Crown Zellerbach Corp., Camas, Washington.

TABLE I. Body Weights of Rats on Diets Containing Conidendrin or Conidendrol.

Compound	Conc. in diet, %	No. of rats	Mean body wt ± S.E.	Exp. minus control ± S.E.	"t"	"P"
			Males			
Control diet	0	9	346 ± 16.8			
α-CNN	.5	9	344 ± 8.0	−2 ± 18.6		
"	1.0	9	359 ± 5.3	13 ± 17.7		
β-CNN	.5	6	327 ± 16.4	−19 ± 23.5		
"	1.0	8	312 ± 12.8	−34 ± 21.1	1.61	
α-CNL	.5	9	353 ± 12.5	7 ± 20.9		
"	1.0	10	349 ± 4.1	3 ± 17.3		
β-CNL	.5	8	353 ± 7.2	7 ± 18.3		
"	1.0	10	307 ± 6.0	−39 ± 17.9	2.18	.05
			Females			
Control diet	0	10	233 ± 6.1			
α-CNN	.125	4	281 ± 14.6	48 ± 15.9	3.02	.01
"	1.0	5	265 ± 9.2	32 ± 11.0	2.91	<.02
β-CNN	.125	5	260 ± 8.1	27 ± 10.2	2.65	.02
"	1.0	5	251 ± 8.9	18 ± 10.8	1.67	
α-CNL	.125	5	232 ± 8.3	−1 ± 10.4		
"	1.0	5	233 ± 9.5	0 ± 11.3		
β-CNL	.125	5	238 ± 9.0	5 ± 10.9		
"	1.0	5	243 ± 4.2	10 ± 7.4	1.35	

S.E. = Stand. error. "t" = Student's t, not recorded unless it is greater than 1. "P" = probability, not recorded unless it is 0.05 or less. CNN refers to conidendrin, CNL to conidendrol. Data for males is for 190 days after the start of the experiment; for females, 200 days.

basic diet consisted of, in percent: corn meal, 73; linseed oil cake meal, 10; alfalfa meal, 2; casein, 10; cod liver oil, 3; bone ash, 1.5; and NaCl, 0.5. The substances under study were added to this basic diet with thorough mixing. Water and food were available *ad libitum*. Concentrations of the substances under study were, for the females, 0.125 and 1.0% of the diet, and for the males, 0.5 and 1.0%. Preliminary experiments suggested that studies of lower concentrations were not necessary. Initially, there were 5 females in each group (except for control group which contained 10 rats), and 10 males per group, 5 animals to each cage. Weekly determinations of body weight, food intake and general appearance were made for 200 days through the rapid growth phase of the animals. Appearance was normal for animals on all of the compounds at all concentrations. Body weights towards the end of the 200-day period are recorded in Table I. (For convenience in calculating, the weights of the males were taken after 190 days on the experimental regimen, while for the females, the time was 200 days.) Statistically, it would appear that one group of males was significantly lighter in weight than the controls, and that 3 groups of females were significantly heavier than their controls. In view of the small number of females in each group, the lack of correlation between dosage levels, and the lack of correlation between sexes, it seems doubtful that the differences are real. Two hundred and twenty days after the start of the feeding, half of the males were autopsied. There was no gross appearance of tissue damage. Organ weights of these animals did not differ significantly from those of control animals of equal body weight, with the possible exception of the livers and kidneys of the rats receiving 1% of α-conidendrol in the diet. For these 2 organs, the livers averaged 16.5 ± 6.3% heavier than controls, and the kidneys, 11.5 ± 5.1% heavier, with P values of about .05.

Results. The remaining animals were continued on their respective diets for an additional 200 days. They appeared normal throughout this period. Hemoglobin values at the end of this 420-day feeding period were within the normal range for the males; hemoglobin values were not determined for the females. Tissues at autopsy appeared grossly normal except for stones in the kidney pelvis of 2 males, one on each dosage level, receiving α-conidendrol, and several cystic kidneys and

yellowish livers in males receiving 1% of β-conidendrol. Tissues from the 420-day animals were prepared for microscopic examination, using a hemotoxylin and eosin stain. The following tissues were examined: heart, lung, liver, spleen, kidney, adrenal, bladder, pancreas, ovary, testis, thyroid, ileum, stomach and, frequently, parathyroid.

In tissues from rats which had received the conidendrins, no changes were found which could be regarded as a direct result of the conidendrin administration. A number of the animals had pneumonia, occasionally with lung abscesses or bronchiectasis, but these animals were in the minority at any dose level. Occasional other changes, such as testicular atrophy, focal pancreatitis, collections of lymphocytes in the kidney and in the heart bore no recognizable relationship to the treatment and were apparently incidental changes.

In the livers of the male rats receiving the higher concentration of β-conidendrol, there was fine vacuolation of the liver cells. In places there were also larger vacuoles. In one animal the change was severe, and there were also small groups of mononuclear cells in the stroma, with minimal increase in fibrous tissue. The change occurred predominantly at the periphery of the lobules. No necrotic cells were identified. Although not specifically tested, the material in these vacuoles was probably fat; this would account for the yellowness of these livers noted grossly. Evidence that severe injury was not associated with this change in liver cells was seen in the form of several mitotic figures in cells containing moderate numbers of fine vacuoles.

A mild similar change was seen in the livers of the female rats which received the larger dose of β-conidendrol. None was seen in the animals on the smaller dose, and only very slight vacuolation of liver cells was seen in 4 of the 10 male animals which received α-conidendrol.

In 3 of the males which received 1% α-conidendrol in the diet, and in 2 on the larger dose of β-conidendrol, there were several small scars in the kidneys, suggesting mild old pyelonephritis. Several of these animals had focal dilatation of renal tubules which contained hyaline casts. One animal which was noted grossly to have a large kidney stone, showed pronounced hydronephrosis. Most of the animals of the male group on these larger doses showed a few more lymphoid cells about the renal pelvis than were present in control animals, but this change was not striking. The kidneys from the females were not distinctly different from controls.

In the females there were no significant changes in the pancreas. In several males on both α- and β-conidendrol there was focal atrophy of acini in the pancreas, sometimes associated with mild local lymphocytic infiltration. These changes could have been results of local duct obstruction, but the observed duct dilatation was not distinctly more than in control animals. A little lymphocytic infiltration of the peripancreatic tissue was sometimes observed. These changes were irregular, without close relationship to the dose of conidendrol.

The other organs showed occasional sporadic changes of various sorts, but nothing which could be attributed to the specific treatment.

The possibility of skin irritation was tested on 4 albino rabbits. Ten per cent ointments of the 4 compounds were prepared by dissolving or suspending the materials in melted Carbowax 1500 W, and stirring while cooling so as to assure uniform distribution in the solidified wax. To each animal approximately 0.1 g of each ointment was applied with gentle rubbing to an area 2 cm in diameter on the clipped skin of the back Except for Saturday and Sunday, applications were made daily for 2 weeks. There was no skin inflammation or other evidence of irritation. At the same time, these ointments were applied to the eye to test for possible irritation of the conjunctiva. Each rabbit received approximately 0.1 g of Carbowax 1500 W in the left conjunctival sac, and an equal amount of one of the ointments in the other eye. These applications were made daily at the same time the skin was being anointed. No conjunctival inflammation was noted in any of the animals. However, a protrusion of the eyeball developed in the right eye of the rabbit receiving

α-conidendrin. This was presumably due to formation of an abscess behind the eyeball. Later, the 3 other animals were treated with the α-conidendrin ointment for 2 weeks, with no adverse reactions being noted. It is doubtful if the protrusion of the eyeball in this rabbit was caused by the ointment.

The possibility of skin sensitization by these compounds was studied in albino guinea pigs by the method of Draize, Woodard, and Calvery(9). Test injections did not indicate sensitization, nor were there any cross reactions when an animal prepared with one of the compounds was tested with the other 3 materials.

The mean maximum human intake of fat, according to *Family Food Consumption and Dietary Levels,* U.S.D.A. Miscellaneous Pub. No. 452, p. 194, 1941, is 2.32 lb/person/week, or somewhat less than 0.4 lb/person/day. If all of the dietary fat were treated with conidendrol at the 0.1% level (which, of course, is not possible since much of the fat is in natural dietary products), the human diet would contain 1/150th as much conidendrol per kg of body weight as was ingested by the rats receiving continuously the 1% diet for half of their normal life-span.

Summary. 1. Weanling rats were fed diets containing one of the following antioxidants: α-conidendrin, β-conidendrin, α-conidendrol, and β-conidendrol. The concentrations were, for the females, 0.125 and 1.0% of the diet; and for the males, 0.5 and 1.0% Body weight increase was normal, or approximately normal, during the first 220 days of the experiment, after which frequent weighings were discontinued. 2. Histological examination of the tissues after 420 days on the diets indicated essentially normal tissues, except for the following: In males receiving the higher concentration of β-conidendrol there was vacuolation (presumably fat) of the liver cells, without indication of severe injury. In several of the males receiving the higher concentration of the conidendrols, there were renal changes suggesting mild, old pyelonephritis. In males, several of the rats receiving the conidendrols had focal abnormalities in the pancreas. 3. There was no evidence of irritation to the skin or to the eye of rabbits, and skin sensitization was not developed in guinea pigs. It is concluded that there is at least a 100-fold margin of safety between the amount of conidendrol suggested for fat antioxidant and the amount ingested by rats eating the 1.0% level.

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