Nonteratogenicity of *Veratrum californicum* in Rabbits

WILLIAM E. JOHNSON and ARNOLD R. MARTIN

Abstract □ An attempt to induce teratogenesis in an inbred colony of New Zealand White rabbits with *Veratrum californicum* has been made. The crude plants were ground and pelleted in a 50-50 mixture with normal rabbit rations and fed to pregnant does for 15 to 24 days of the gestation period. The incidence of fetal malformation and resorptions was found to be not significantly different than the established incidence for the colony.

Keyphrases □ *Veratrum californicum*—nonteratogenicity, rabbits □ Feeding study, rabbits—*V. californicum*

Considerable interest has developed in the capacity of western hellebore (*Veratrum californicum*) to provoke congenital deformity in lambs from ewes grazing upon, or experimentally fed, the leaves and stems of the plant. Head deformities with distortion or absence of some facial bones, cyclopian deformity of the eyes, cebocephalus, microphthalmia, and anophthalmia were reported in 1964 (1). Fusion of the cerebral hemispheres, absence or displacement of the pituitary, hydrocephalus, and leg deformities were also noted. Subsequent work has determined that gestation Day 14 is the critical period of vulnerability and no abnormal lambs were produced when ewes were fed the plant on Days 11, 12, 13, 15, or 16 (2). In the search for the active teratogenic ingredient or ingredients from *V. californicum*, treatment of ewes with veratramine, a Veratrum alkaloid, resulted in a 37.5% incidence of deformity (3). An unexpected feature of the teratogenicity was the absence of cyclopia which had been routinely observed whenever the crude leaves and stems were used. The veratramine syndrome included bowing of front limbs, knee joint flexture, and flaccid skeletal muscle. In 1967 it was reported that the Veratrum-induced type of malformations could be caused in lambs by stomach tube feeding of ground Veratrum plants or its roots to ewes on Day 14 of gestation (4). A preliminary identification of veratramine in the Veratrum plant material under investigation was also reported.

The current high interest in teratogenic substances in human medicine lends added interest to these reports. Veratrum alkaloids have been used for a number of years in cardiovascular disorders in humans. This report deals with an attempt to induce teratogenesis in New Zealand White rabbits with *V. californicum*. The economic advantage of a smaller laboratory animal over sheep for teratogenic studies is obvious.

EXPERIMENTAL

Stems and leaves of *V. californicum* were collected in early July in Wallowa County, Oregon and in the immediate vicinity of Pullman, Washington. Plants were harvested in the early flowering stage and quickly dried in direct sunlight. The dried plants were ground, mixed with normal rabbit ration, and pelleted in such a way that the Veratrum material constituted 50% by weight of the finished pellets. New Zealand White does from a local inbred colony were grouped and treated as follows:

Group 1 (5 does) were fed the Veratrum ration for 13 days without breeding to determine the capacity to maintain body weight on the unusual feed.

Group 2 (5 does) were given only normal feed, bred, and examined on Day 28 of gestation.

Group 3 (5 does) were fed the Veratrum ration for 15 days starting on the third postcoital day.

Group 4 (3 does) were fed the Veratrum ration for 24 days starting on the third postcoital day.

At 28 days postcoitum each doe was sacrificed with marginal ear vein air injection. The entire uterus was quickly removed and the young were observed for normal spontaneous motor activity. An inspection was made for external fetal abnormalities and resorption sites. The heart, kidney, intestinal tract, lungs, liver, brain, palate, tongue, and orbits were examined for gross anatomic abnormalities.

The internal organs were removed from each fresh fetus and examined for gross abnormalities of structure and general appearance. Free-hand section of each organ was performed and the tissue sections were visually examined with the aid of a dissecting microscope.

RESULTS AND DISCUSSION

Control Group 1 had an average body weight of 3.455 kg. immediately before they were started on Veratrum pellets. After 13 days on the Veratrum pellets the average body weight was 3.237 kg. No animal of the group showed signs of ill health during the course of the experiment. Their appetite for the unusual feed was less than for normal feed. Control Group 2 produced 43 normal offspring and no abnormalities or resorptions were observed. Other more extensive studies on this same colony of New Zealand White rabbits (230 kits) have led to a determination of 0.9% for spontaneous malformations and 2.7% for resorptions. Results of experiments with Veratrum are tabulated in Table I.

It is quite apparent from the results of this work that the New Zealand White colony of rabbits under investigation are not highly vulnerable to the tertatogenic effects of *V. californicum*. The one instance of fetal malformation from a doe which had been fed Veratrum ration for 24 days during her gestation period involved severely shortened ribs on one side. The failure of the extended (24 days) Veratrum feeding period to cause resorptions minimizes the significance of the approximately 6% resorption incidence in the 15-day group. Tests for significance of difference between groups within the experimental design were made according to the method of significance of difference in proportions of samples from a normal distribution (5). p value calculated for significance of difference in

Table I—Incidence of Fetal Malformations and Resorptions in *Veratrum californicum*-Treated Rabbits

Treatment	Number of Does	Number of Kits	Number of Malformations	Number of Resorptions	Muscle Tone
Normal feed (Group 2)	5	43	0	0	Normal
15-day Veratrum ration (Group 3)	5	50	0	3	Normal
24-day Veratrum ration (Group 4)	3	27	1	0	Normal

resorptions in Group 3 *versus* Control Group 2 equals 0.73, in malformations in Group 4 *versus* Control Group 2 equals 0.39, and in malformations in Group 4 *versus* Group 3 equals 0.39. In any event the incidence of malformations and resorptions is not significant compared to the rate of spontaneous fetal failures in this rabbit colony. It can be concluded from the study that this strain of New Zealand White rabbits is not a suitable animal for further investigation of the teratogenicity of *V. californicum*. This conclusion need be qualified by the report that the toxicity and teratogenic agents in *V. californicum* have shown variation between range areas with regard to activity in the ewe (1).

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New Compounds: Acrylonitrile Derivatives as Potential Antineoplastic Agents

EDWARD O. MAGARIAN* and W. LEWIS NOBLES†

Abstract □ The synthesis of several heterocyclic acrylonitriles and the corresponding carboxylic acid derivatives is described.

Keyphrases □ Acrylonitriles, heterocyclic—synthesis □ Carboxylic acid derivatives, acrylonitriles—synthesis

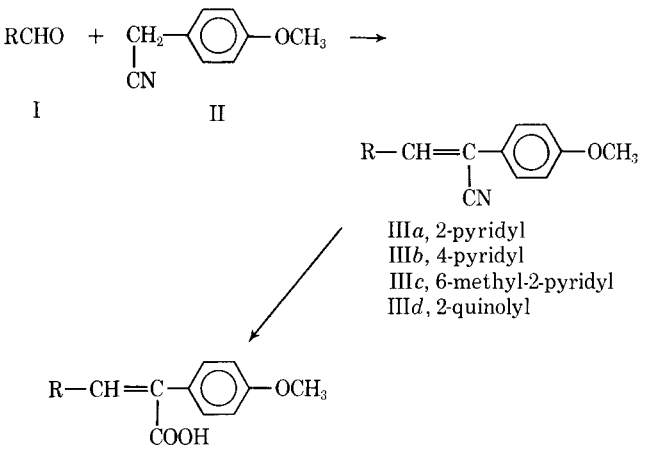
Estrogens have proven to be of clinical value in the treatment of prostatic and breast carcinomas (1). It has been shown that many acrylonitrile derivatives possess excellent estrogenic activity (2). As part of the continuing search for newer and more effective antineoplastic agents, several acrylonitriles and their acid derivatives were prepared for biological evaluation.

The synthesis of 2-(*p*-methoxyphenyl)-3-(4-pyridyl)-acrylonitrile(III*b*) was initially attempted in pyridine but only the corresponding acid, 2-(*p*-methoxyphenyl)-3-(4-pyridyl)acrylic acid (IV*b*), was obtained. Subsequently, it was discovered that III*b*, as well as the other nitriles, could be conveniently prepared by modifying the procedure of Castle and Seese (3). The appropriate heterocyclic aldehyde (I) was allowed to condense with *p*-methoxyphenylacetonitrile (II) under basic conditions (Scheme I).

The other carboxylic acids (IV*a*, *c*, *d*) were prepared by hydrolyzing the nitriles in aqueous sulfuric acid.

EXPERIMENTAL[1]

2-(p-Methoxyphenyl)-3-(2-pyridyl)acrylonitrile (IIIa, Table I)—The procedure described for the preparation of this compound is typical. To a solution of 3.0 g. (0.020 mole of *p*-methoxyphenylacetonitrile and 2.0 g. (0.022 mole) of 2-pyridinealdehyde in 40 ml. of absolute methanol was slowly added an absolute methanol solution of sodium methoxide (0.5 g., 0.022 g.-atom of sodium metal in 10 ml. of absolute methanol). The mixture was heated at 50–60° for 5–10 min., then allowed to stand at room temperature for 30 min., and finally cooled in an ice bath. The solid was removed by filtration, air-dried, and recrystallized from ethanol.



Scheme I

2-(p-Methoxyphenyl)-3-(6-methyl-2-pyridyl)acrylic acid (IVc, Table I)—The preparation of the title compound according to the following procedure may be taken as typical. To 48 ml. of concentrated sulfuric acid in 65 ml. of water was added 6.0 g. (0.027 mole)

[1] Melting points were determined on a Thomas-Hoover Uni-melt apparatus and are uncorrected. Elemental analyses were performed by Dr. Alfred Bernhardt, 433 Mulheim (Ruhr), Hohenweg 17, West Germany. For physical and analytical data see Table I.