ciate the engravings with a particular cultural tradition because they are associated with both the Wilton and the Oakhurst complexes represented in the Holocene sequence at Wonderwerk Cave. That rock painting can similarly not be associated with a particular tradition has also been demonstrated by the observation that paintings are found not only in association with the Wilton Complex dating to the Holocene but also in association with different and older material dating to the Upper Pleistocene (*1*, *2*). When larger samples of dated rock art become available, it may be possible to make stylistic distinctions between cultural traditions.

Rock art in South Africa has been variously interpreted as either representational art (*16*) or as expressions of ritual and symbolism (*17*, *18*). There is good reason to assume that the artists responsible for both paintings and engravings shared at least some common beliefs. Quantitative analyses of rock art drawn from relatively large samples indicate that the eland is the predominant animal represented in both paintings (*17*) and engravings (*19*). Therianthropes (animal-headed figures) are similarly found in both media, although they are rare in engravings (*20*). Attention has been drawn to the possibility that some paintings in the Drakensberg region may represent an expression of trance experience and beliefs associated with rituals similar to those known among modern !Kung Bushmen (*18*). Recent studies on human subjects show that grid systems and parallel lines of the kind represented in most of the Wonderwerk engravings are often typical of hallucinatory experience (*21*). However, grids and line systems are often used for figure infilling and portion emphasis in engravings, or even to depict the ground surface (*20*). As all except one of the Wonderwerk engravings are broken, it is not possible to establish whether the grid and line depictions are examples of representational art or are best interpreted in terms of concepts associated with trance experiences.

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6-Methoxybenzoxazolinone: A Plant Derivative That Stimulates Reproduction in *Microtus montanus*

Abstract. *A plant-derived cyclic carbamate, 6-methoxybenzoxazolinone, that stimulates reproductive activity in* Microtus montanus *has been isolated. This nonestrogenic compound may be a naturally occurring environmental cue affecting reproductive cycles in many mammals.*

More than 40 years ago, Rowan (*1*) noted that endocrine systems regulating mammalian reproduction respond to an environmental stimulus, the photoperiod. In 1946, Bodenheimer made the observation that major outbreaks of voles in Palestine could not be correlated with prevailing environmental factors such as climate, photoperiod, volume of food resources, or population density, acting either alone or in combination. From his studies, Bodenheimer suggested that vole outbreaks were associated with the action of an unknown factor in the food supply (*2*). Negus *et al.* (*3*) also observed a correlation between reproductive activity in the montane meadow vole, *Microtus montanus*, and the onset of growth of the plant food resource. Subsequent field and laboratory experiments confirmed the existence of a plant factor that increases fertility and triggers reproductive activity in *M. montanus* (*4*, *5*).

Microtus montanus typically inhabits the mesic montane meadows of western North America. The onset and termination of the growing season in these environments may vary by 1 month or more from year to year. Thus the vegetative food resources are highly uncertain for a short-lived herbivore. Accordingly, if an environmental cue were available to *Microtus* that accurately predicted food resources in the near future, optimal timing of reproductive effort could be attained with the attendant benefits to fitness (*6*). Our studies of responses by *Microtus montanus* to green plant material in the diet have led us to the identification of a naturally occurring compound that functions as a reproductive trigger in this species.

Winter wheat was used for extraction because previous studies indicated that an acetone-ether wheat extract was effective in stimulating reproduction in *Microtus montanus* (*4*, *5*). The wheat was grown under standard greenhouse conditions to a height of 10 cm, harvested, and homogenized with an equal weight of acetone. The mass was suction-filtered, and the filtrate, including several acetone washes, was freed from acetone at room temperature by means of an aspirator. The remaining heteroge-

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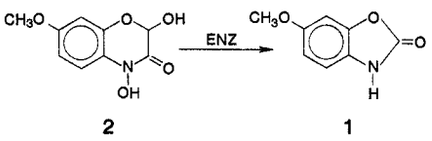

Fig. 1. Chemical structure and enzymatic conversion of DIMBOA (**2**) to 6-MBOA (**1**).

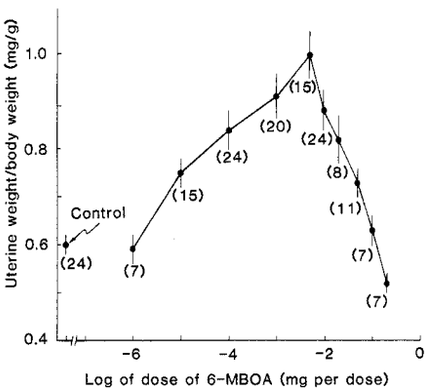

Fig. 2. Uterine weight response of subadult *Microtus montanus* to varying doses of 6-MBOA.

neous mixture was extracted with pentane. The resulting straw yellow aqueous solution was extracted repeatedly with diethyl ether until all color was removed. After the combined ether extracts were reduced in volume on a rotary evaporator, a light brown oil was left. Part of this oil was bioassayed for activity (*7*) and the rest was submitted to fractionation procedures. The amount of oil freed from basic compounds by acid extraction averaged 150 mg per kilogram of fresh wheat sprouts. It was silylated with bis-silylacetamide and partitioned by gas chromatography (*8*) into four fractions. Two of these exhibited stimulatory activity. Since one of the active fractions was composed of a single chromatographic peak, it was examined first and found to be composed of almost pure silylated *cis*- and *trans*-aconitic acids.

The mass spectrum of this substance revealed an impurity which proved to be almost identical with that of a component of the other stimulatory group. The component, which was collected by gas chromatography as a pure fraction, was an unusual silyl derivative. When collected directly from the gas chromatograph under an inert atmosphere, the derivative demonstrated a strong infrared absorption at 2220 cm^{-1}. Upon exposure to moisture, the absorption at 2220 cm^{-1} disappeared and a new one appeared at 1770 cm^{-1}. The only structure that could easily explain these results was an isocyanate with an adjacent silyl ether (*9*). The silyl ether would hydrolyze on exposure to moisture and then react with the isocyanate to produce a cyclic carbamate. The presence of absorption in the infrared data suggested that the cyclic carbamate was a 2-benzoxazolinone. A search of the literature for naturally occurring 2-benzoxazolinones revealed that the infrared spectrum of 6-methoxybenzoxazolinone (6-MBOA) (Fig. 1, structure **1**) was identical with that of the compound that had been isolated (*10*). An authentic sample of 6-MBOA was synthesized (*11*) and observed not to depress the melting point when mixed with the isolated compound. However, 6-MBOA gave negative results when bioassayed at 10, 5, and 2 mg per gram of laboratory diet.

An examination of various methods of concentrating the stimulatory factor revealed that the activity of the extract could be removed by lead complex precipitation and regained by decomposing the complex with hydrogen sulfide. The yellow oil extracted with diethyl ether from the aqueous solution of the freed complex was fractionated by dry column chromatography (*12*) to give an active fraction, which proved to be composed primarily of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one (DIMBOA) (Fig. 1, structure **2**). This compound and its naturally occurring glycosides are biochemical precursors of 6-MBOA (*13*). With the evidence again indicating that 6-MBOA was the stimulatory compound, a wide range of concentrations of 6-MBOA was examined for activity. Strong stimulatory activity was observed between 0.02 and 0.1 mg per gram of Chow, whereas smaller or larger doses were relatively ineffective. A dose-response curve was obtained by administering 6-MBOA intraperitoneally while the uterine weight response was monitored (*14*) (Fig. 2). A threshold of response occurred at 0.01 μg of 6-MBOA per day for three consecutive days. The response is maximal at 5 μg per day, larger doses being less effective and becoming slightly inhibitory at the highest dose tested (200 μg per day).

Several studies were carried out to determine whether 6-MBOA was the reproductive stimulator that had been hypothesized (*4, 5*). Berger and Negus (*15*) had determined by ovariectomy that the stimulator compound was not estrogenic. Administering 6-MBOA to ovariectomized juvenile *Microtus* likewise failed to stimulate an increase in uterine weight (*16*) (Table 1). To examine the effect of 6-MBOA on ovarian response in a spontaneous ovulator, we injected laboratory mice (Swiss Webster) intraperitoneally with a single dose (100 or 250 μg) and killed them 24 hours after injection.

Table 1. Dietary effects of 6-MBOA on the uterine weight of sham-operated (S) and ovariectomized (O) juvenile *Microtus montanus*. M, diet supplemented with 6-MBOA; NM, without supplement. Results are expressed as the mean ± standard deviation.

Treatment	*N*	Body weight (g)	Uterine weight/body weight (mg/g)
S-NM	4	19.0 ± 1.7	0.48 ± 0.09
S-M	8	19.6 ± 1.2	0.61 ± 0.09*
O-NM	8	20.6 ± 1.0	0.45 ± 0.05
O-M	7	20.1 ± 1.1	0.43 ± 0.04

*Significantly different from all treatments, $P < .025$

Table 2. Effects of 6-MBOA on ovarian weight in white mice. Results are expressed as the mean ± standard deviation.

6-MBOA (mg)	*N*	Body weight (g)	Ovarian weight (mg)
0	14	32.5 ± 3.1	12.6 ± 2.8
100	9	35.1 ± 5.2	19.2 ± 8.6*
250	24	34.7 ± 3.6	16.9 ± 5.6†

*Significantly different from control at $P < .025$.
†Significantly different from control at $P < .005$.

There was a significant increase in ovarian weight in animals receiving 6-MBOA in comparison with control animals (Table 2). Histological examination of the ovaries established that much of the increase in weight was due to an increase in the number of large antral follicles. A similar ovarian response was observed in *M. montanus* receiving a dietary supplement of spinach extract (*5*). The uterine weight response, the ovariectomy data, and the ovarian follicle growth response indicate that 6-MBOA is the reproductive stimulator previously hypothesized. Field studies on natural populations have further confirmed that 6-MBOA is the plant compound that triggers reproduction in *M. montanus* (*17*).

The isolation and identification of 6-MBOA is a demonstration of a specific chemical that functions as a cue to initiate reproduction in a mammalian system. We previously reported the identification of a group of compounds (cinnamic acids) from mature grasses that may function to signal the end of the vegetative growing season and therefore the deterioration of the plant food resource for *M. montanus* (*18, 19*). For *M. montanus*, both 6-MBOA and the cinnamic acids appear to define when a high-quality food supply will be available to support a breeding effort. Such definition of the food resource is critical for species inhabiting uncertain environments. Whether this mechanism of environmen-

tal interpretation is peculiar to *M. montanus* remains to be determined. However, the ovarian response of the laboratory mouse suggests the potential of a more general mammalian response to chemical cues in the food resource.

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7. The plant fractions to be assayed were dissolved in a 1:4 mixture of ethanol and ether and mixed with ground Purina Laboratory (Rat) Chow; the solvent was allowed to evaporate. Control animals received Chow coated with solvent only. The assay animals, juvenile *Microtus montanus* (17 to 19 days old and weighing 13.0 to 14.5 g), were caged in pairs and given free access to the experimental or control diets. After a 7-day feeding period, the animals were killed and the uteri were removed and weighed.
8. A gas chromatograph (Perkin-Elmer 880) equipped with a flame ionization detector was used to separate components on a glass column (6 feet by 1/8 inch) packed with 3 percent OV-17 on Chromosorb AW-DMCS, 80/100 mesh. The column oven was programmed to increase from 80° to 200° C at a rate of 4° C per minute.
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Chemical Triggering of Reproduction in *Microtus montanus*

Abstract. *In a replicated experiment, nonbreeding winter populations of* Microtus montanus *were given supplements of rolled oats coated with 6-methoxybenzoxazolinone, a naturally occurring plant derivative. After 3 weeks of this feeding regime, samples from the populations demonstrated a high incidence of pregnancy in females and testicular hypertrophy in males. Control populations receiving rolled oats coated only with the solvent showed no reproductive activity. These results demonstrate that the presence of 6-methoxybenzoxazolinone in the plant food resource acts as the ultimate cue to trigger reproductive effort in* Microtus montanus.

In 1977, we presented field experimental evidence that the montane vole (*Microtus montanus*) is cued to its reproductive effort by chemicals in the plant food resources (*1*). Subsequently, we were successful in isolating and identifying the active chemical component (*2*). Responses to 6-methoxybenzoxazolinone (6-MBOA) were directly comparable to those elicited from fresh grass supplements and laboratory experiments with crude extracts (*3*). However, the ultimate proof of the efficacy of 6-MBOA in triggering reproduction remained to be demonstrated in the field. We now present experimental proof that 6-MBOA is responsible for triggering reproduction in *Microtus montanus*.

Replicated field experiments were accomplished in the winters of 1978–79 and 1979–80, in the salt grass meadows (*Distichlis stricta*) at Timpie Springs, Utah. Previous triggering experiments had been performed at the identical site (*1*). Experimental and control plots were selected as described (*1*, *4*). Before each experiment, the *Microtus* population was sampled by livetrapping (Table 1); these samples demonstrated that the populations were reproductively inactive. We then provided supplemental food in the experimental and control plots. In the experimental plots, the supplemental food consisted of rolled oats coated with 6-MBOA (40 μg per gram of oats) (*5*). The supplemental food for the control plot was rolled oats coated with solvent only. Thus, the only difference between the supplemental treatments in the two plots was the addition of 6-MBOA in the experimental plot. Oats were distributed in each plot at the rate of 1 kg every 7 days for 3 weeks. Petri dishes containing the oats were placed beside runways; the food was protected from weather by galvanized metal shelters (*6*).

When the supplemental feeding period was terminated, both areas were sampled by livetrapping, and the animals were killed and examined for reproductive activity (*7*). In the winter of 1978–79, the experimental regime was initiated on 13 January 1979 and terminated on 11 February. In the winter of 1979–80, a similar experiment was initiated on 24 November 1979 and terminated on 17 December. The rationale for the earlier timing of the second experiment was to determine whether there was a period before the winter solstice (22 December) when the population would be unresponsive to 6-MBOA. The two experimental regimes produced essentially identical results (Table 1).

In both experiments, a large propor-

Table 1. Reproductive responses of female and male *Microtus montanus* to supplements of 6-MBOA coated on rolled oats (E) during January and February 1979 (experiment 1) and November and December 1979 (experiment 2), compared with responses of control animals (C) that received supplements of rolled oats coated only with the solvent.

Date	Sex	Treatment	*N*	Proportion pregnant	Weight of uterus or testes (mg)
		Experiment 1			
4 December 1978	♀	Presample	6	0.0	4.6 ± 1.5
11 February 1979	♀	E	11	0.6	58.6 ± 8.0*
11 February 1979	♀	C	11	0.0	32.7 ± 18.2
11 February 1979	♂	E	11		231.6 ± 55.8
11 February 1979	♂	C	8		139.2 ± 76.6
		Experiment 2			
29 October 1979	♀	Presample	8	0.0	10.6 ± 5.4
18 December 1979	♀	E	7	0.86	40.1 ± 8.7*
18 December 1979	♀	C	22	0.0	14.2 ± 7.9
18 December 1979	♂	E	9		137.2 ± 35.1
18 December 1979	♂	C	10		63.2 ± 57.2

*Includes only the uterine weights of animals not visibly pregnant.

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