climatic change perhaps an order of magnitude less than the difference between full-glacial, pluvial woodland and postglacial, desert conditions.

Assuming that the $\delta^{13}C$ values of the samples > 40,000 and 10,000 years old are indicative of their photosynthetic mechanism and water-use efficiency, then these results provide further evidence of increasing dryness of the Nevada site. The presence of the C_4 species of *Atriplex* at both times is evidence that conditions throughout the entire period are likely to have been relatively arid. Intracellular resistance to CO_2 exchange is lower in C_4 plants than C_3 plants, which is apparently responsible for the higher water-use efficiency in C_4 plants (*10*). The evidence that the C_4 species, *Atriplex confertifolia*, was much more abundant in the 10,000-year-old deposit can be taken as an indication of especially warm and dry conditions toward the close of the last major glacial stage of the Pleistocene. Presumably, the shift from C_3-like to C_4-like carbon isotope ratios in the CAM plant (*Opuntia polyacantha*), also indicates that the Frenchman Flat area was already more arid about 10,000 years ago, even a millennium or more before the local demise of the pluvial juniper woodlands.

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$$\delta^{13}C \text{ (per mil)} = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{PDB}} - 1\right] \times 1000$$

where the PDB standard is the belemnite from the Peedee Formation in South Carolina.
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12. The leaf material was collected from cave deposits and carbon dated by using procedures described by Wells and Berger (*3*). There is a possibility of some contamination of the sample by carbon from other sources, but in the work reported here there was no pretreatment of the sample, and the results suggest that it may not be essential for the object of this study. The material was burned to completion in an oxygen atmosphere and all CO_2 was recovered free from contamination. The $^{13}C/^{12}C$ ratio was measured in a mass spectrometer, with appropriate corrections for effects of other isotopes on mass 44 and 45 and instrument effects. The value was expressed as $\delta^{13}C$; an accuracy of ± 0.1 per mil is expected.
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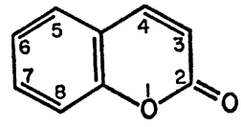
Genetic Variation in Coumarin Hydroxylase Activity in the Mouse (Mus musculus)

Abstract. *Basal and phenobarbital-induced rates of hepatic metabolism of coumarin to 7-hydroxycoumarin are markedly higher in DBA/2J mice than they are in the AKR/J, C57BL/6J, and C3H/HeJ strains. Intermediate coumarin hydroxylase activity in F_1 hybrids of mating between DBA/2J and the other three strains indicates an additive mode of inheritance.*

Studies with different strains of the same animal species (*1*) and family studies in man (*2, 3*) indicate significant genetic regulation of microsomal mixed-function oxidases that metabolize drugs, carcinogens, and other foreign chemicals (*4*). Working with inbred strains of mice, Thomas *et al.* (*5*) and Nebert and his associates (*6*) have shown that induction of benzo[*a*]pyrene hydroxylase (aryl hydrocarbon hydroxylase) by 3-methylcholanthrene and other polycyclic hydrocarbons is principally determined by a single autosomal dominant gene. We now describe genetic control of basal and phenobarbital-induced levels of a microsomal mixed-function oxidase that hydroxylates coumarin (see structure), a naturally occurring constituent of many plants and the nucleus for the coumarin oral anticoagulant drugs.



Coumarin

Male mice of four inbred strains and three F_1 hybrids, 6 to 7 weeks old, were purchased from the Jackson Laboratory, Bar Harbor, Maine. Hepatic metabolism of coumarin to 7-hydroxycoumarin was determined spectrofluorometrically by a modification of the method of Creaven *et al.* (*7*). Incubation mixtures, in a final volume of 1.0 ml, contained 2 mg (wet weight) of liver, 50 μmole of tris-HCl (*p*H 7.5), 250 μmole of sucrose, 3 μmole of $MgCl_2$, 0.5 μmole each of the reduced forms of nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NADH), and 1.0 μmole of coumarin.

Basal and induced coumarin hydroxylase activities in four inbred mouse strains and three F_1 hybrids are summarized in Table 1. The data indicate that enzyme activity in noninduced DBA/2J mice is three- to fourfold higher than in AKR/J, C57BL/6J, or C3H/HeJ mice. After 3 days of phenobarbital treatment, the relative difference in enzyme activity between DBA/2J and the other three strains is four- to sixfold. 7-Hydroxycoumarin formation in liver homogenates of F_1 hybrids of matings between DBA/2J mice and the other three strains is intermediate in both untreated animals and those treated with phenobarbital, indicating additive inheritance. Table 1 also shows the lack of induction of coumarin hydroxylase activity by 3-methylcholanthrene (3-MC) in any of the inbred or hybrid mice. In agreement with published results (*5, 6*) benzo[*a*]pyrene hydroxylase activity in liver homogenates from the same ani-

Table 1. Basal and induced coumarin hydroxylase activity in the livers of some inbred and hybrid mice. Sodium phenobarbital was administered intraperitoneally for 3 days prior to the time the mice were killed (daily dose, 75 mg/kg). 3-Methylcholanthrene (100 mg/kg), dissolved in corn oil, was administered intraperitoneally 24 hours prior to the time of killing. Each value represents the mean ± the standard error of the mean of enzyme activity from three mice. Values are expressed as picomoles of 7-hydroxycoumarin formed per minute per milligram (wet weight) of liver.

Mouse strain	Coumarin hydroxylase		
	Control	Phenobarbital treated	3-Methylcholanthrene treated
DBA/2J	54 ± 12	239 ± 20	38 ± 7
AKR/J	16 ± 4	38 ± 2	13 ± 3
C57BL/6J	14 ± 1	59 ± 4	15 ± 2
C3H/HeJ	17 ± 2	45 ± 0.4	19 ± 6
$AKD2F_1/J$	23 ± 1	135 ± 5	22 ± 2
$B6D2F_1/J$	27 ± 2	128 ± 14	25 ± 3
$C3D2F_1/J$	24 ± 3	103 ± 4	26 ± 4

Table 2. Coumarin hydroxylase activity, cytochrome P-450 content, and cytochrome c reductase activity in two inbred mouse strains and the F_1 hybrid treated with phenobarbital. In each experiment four animals of each strain received sodium phenobarbital (1 mg/ml) in their drinking water for 8 days. Two animals received a single intraperitoneal dose of coumarin (2.0 μmole), and their urines were collected for 24 hours. After dilution of the pooled urine to 50 ml with distilled water, samples were incubated for 2 hours at 37°C with 0.10 ml of Glusulase (*15*) to convert conjugated 7-hydroxycoumarin (7-HC) to its unconjugated form. Incubations without Glusulase indicated that 80 to 90 percent of urinary 7-HC was conjugated. Animals not receiving coumarin were utilized for the determination of enzyme activities and cytochrome P-450 content.

Assay	Strain DBA/2J		Strain AKR/J		Strain $AKD2F_1/J$	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Urinary 7-HC*	765	821	36	27	106	190
Coumarin hydroxylase†						
Liver	791	601	42	62	430	221
Lung	1.9	7.1	0.3	0.3	0.7	2.2
Kidney	0.3	1.7	0	0.1	0.2	0.3
Cytochrome P-450‡	2.91	2.91	2.42	2.75	2.53	2.72
Cytochrome c reductase§	378	287	335	283	319	263

* Nanomoles of 7-HC excreted in 24 hours after a single intraperitoneal dose of coumarin (2 μmole). † Activity expressed as picomoles of 7-HC formed per minute per milligram (wet weight) of tissue. ‡ Nanomoles per milligram of liver microsomal protein, as determined by the method of Omura and Sato (*16*). § Nanomoles of cytochrome c reduced per minute per milligram of liver microsomal protein, as determined by the method of Phillips and Langdon (*17*).

mals treated with 3-MC was induced four- to fivefold in C57BL/6J and C3H/HeJ but not at all in DBA/2J or AKR/J strains. It is thus apparent that benzo[*a*]pyrene hydroxylase induction and coumarin hydroxylase induction are under separate regulatory control.

Differences in the level of hepatic coumarin hydroxylase activity among AKR/2J, DBA/2J, and $AKD2F_1/J$ mice pretreated orally with phenobarbital reflect the extent of in vivo metabolism of coumarin to 7-hydroxycoumarin and its excretion in the urine (see Table 2). Urinary excretion of 7-hydroxycoumarin for 24 hours after a single intraperitoneal dose of coumarin is 25 times higher in DBA/2J mice than it is in AKR/J mice, while liver coumarin hydroxylation is 14-fold higher. Although the lung and kidney had less than 1 percent of liver coumarin hydroxylase activity, the data indicate a higher hydroxylase activity in these extrahepatic tissues of DBA/2J mice than in the AKR/J strain. The F_1 offspring show intermediate levels of 7-hydroxycoumarin excretion, as well as hepatic and extrahepatic enzyme activity. Skin and small intestine had undetectable levels of activity in both strains and in the F_1 hybrid.

Cytochrome P-450, the terminal electron acceptor of the microsomal mixed-function oxidases, and cytochrome c reductase, which reduces cytochrome P-450 with electron equivalents donated from NADPH, are both inducible by phenobarbital (*4*). The data in Table 2 indicate that levels of microsomal cytochrome P-450 and cytochrome c reductase in liver are not significantly different in the two mouse strains and in the F_1 hybrid. As the uninduced levels of these two components were not significantly different in DBA/2J, AKR/J, and $AKD2F_1/J$ mice (data not shown), a preferential synthesis or induction of total cytochrome P-450 or cytochrome c reductase cannot account for the increased rate of metabolism of coumarin in DBA/2J mice. However, if there are several types of cytochrome P-450 molecules, a preferential synthesis in DBA/2J mice of a type with an increased ability to hydroxylate coumarin might explain our findings.

We have also found that DBA/2J mice are less sensitive than AKR/J mice are to the lethal effects of the coumarin oral anticoagulants warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] and Dicumarol (bishydroxycoumarin; 3,3′-methylenebis[4-hydroxycoumarin]) administered in the diet up to 15 days. Whether the decreased sensitivity to anticoagulants in DBA/2J mice is related to the rapid hydroxylation of coumarins in this strain remains to be determined. It should be noted that the principal metabolic fate of coumarin (*8*) and warfarin (*9*) in man is hydroxylation at the 7 position of the aromatic ring, and Dicumarol is believed to be inactivated by aromatic ring hydroxylation (*2*). The induction of coumarin anticoagulant metabolism in man, as well as in the rat, by phenobarbital has also been well documented (*10*).

The rate of metabolism of Dicumarol and several other drugs is under genetic control in man (*2*, *3*). The variation in plasma half-lives of Dicumarol and other drugs studied, such as antipyrine and phenylbutazone, appears to be transmitted as polygenetically controlled traits. Our studies, in which we used backcross and intercross mating of DBA/2J and AKR/J mice and their offspring, indicate that coumarin hydroxylase activity is inherited as an autosomal trait controlled principally by a single gene locus (*11*).

Since coumarin and the coumarin-derived oral anticoagulants are metabolized by analogous reactions in mouse and man, perhaps coumarin hydroxylase activity in a readily obtainable human tissue, such as skin or lymphocytes, could predict the in vivo metabolism or half-lives of the coumarin oral anticoagulants. Recent studies (*12*) have shown that cultured human blood lymphocytes contain the microsomal mixed-function oxidase system. Using these human cells, Kellerman and his associates have shown that the inducibility of benzo[*a*]pyrene hydroxylase by polycyclic hydrocarbons is genetically determined in man, as in the mouse, and is inherited as a single autosomal trait (*13*). The positive correlation between high benzo[*a*]pyrene hydroxylase inducibility in lymphocytes and bronchogenic carcinoma (*14*) suggests that measurement of the enzymatic metabolism of foreign chemicals in suitable human cells may predict the in vivo metabolism and action of these chemicals.

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Sex Pheromones and Reproductive Isolation of the Lesser Peachtree Borer and the Peachtree Borer

Abstract. *(E,Z)-3,13-octadecadien-1-ol acetate, and (Z,Z)-3,13-octadecadien-1-ol acetate, isolated from the female lesser peachtree borer,* Synanthedon pictipes *(Grote and Robinson), and the female peachtree borer,* Sanninoidea exitiosa *(Say), respectively, strongly attract the respective males of these species in field bioassays. These compounds are the largest pheromones isolated thus far from a lepidopterous species.* Sanninoidea exitiosa *males did not respond to the synthesized* (E,Z)*-isomer, and low concentrations of it in the synthesized* (Z,Z)*-isomer did not interfere with their response to the* (Z,Z)*-isomer. In contrast, even very low concentrations of the* (Z,Z)*-isomer (1 percent) in the* (E,Z)*-isomer significantly inhibited the response of* Synanthedon pictipes *males.*

At least three examples of reproductive isolation of closely related lepidopteran species by pheromones have been described. Male *Trichoplusia ni* (Hübner), the cabbage looper, and *Autographa californica* (Speyer), the alfalfa looper, respond to different concentrations of the same pheromone, (*Z*)-7-dodecen-1-ol acetate (*1*). Males of two tortricid moth species, *Adoxophyes orana* (F.v.R.) and *Clepsis spectrana* (Treitschke), respond to the different relative concentrations of (*Z*)-9- and (*Z*)-11-tetradecen-1-ol acetate produced by the females of these species (*2*). Females of the Indian meal moth, *Plodia interpunctella* (Hübner), and of the almond moth, *Cadra cautella* (Walker), both produce the same pheromone, (*Z,E*)-9,12-tetradecadien-1-ol acetate (*3*). However, the almond moth female produces a second compound, (*Z*)-9-tetradecen-1-ol acetate, that stimulates almond moth males and inhibits Indian meal moth males (*4*), and the Indian meal moth female produces a second compound, (*Z,E*)-9,12-tetradecadien-1-ol, that inhibits the response of almond moth males (*5*).

Release-recapture tests with the lesser peachtree borer, *Synanthedon pictipes* (Grote and Robinson), and the peachtree borer, *Sanninoidea exitiosa* (Say) (species that occur east of the Rocky Mountains in North America from the Great Lakes to Florida), have indicated that pheromones may be useful in controlling these pests (*6*). When marked *S. pictipes* males were released in an Indiana peach orchard, 82 percent were recovered in 2 days. The larvae of these species feed on the cambium tissue of the tree and severely damage peach trees, other fruit trees, and several woody ornamentals.

We now report the isolation, identification, and synthesis of the sex pheromones of the lesser peachtree borer and the peachtree borer. The synthesized pheromone of *S. pictipes*, (*E,Z*)-3,13-octadecadien-1-ol acetate, was strongly attractive to males of this species in field tests but did not attract *S. exitiosa* males. Indeed, when it was mixed in a 1 : 1 ratio with the synthesized pheromone of *S. exitiosa*, (*Z,Z*)-3,13-octadecadien-1-ol acetate, it appeared to inhibit the response of *S. exitiosa* males. Similarly, the synthesized *S. exitiosa* pheromone was strongly attractive to *S. exitiosa* males in the field. However, when the (*Z,Z*)-isomer was present in only 1 percent concentration in the *S. pictipes* pheromone, it significantly inhibited the response of *S. pictipes* males. Thus, the two species are isolated reproductively by the male responses to two geometrical isomers of the same compound. Additionally, the strong inhibition of the response of males of one species by the pheromone of the other species reinforces their isolation and may provide a useful mechanism (disruption of the communications system) for control of these insects (*7*).

The initial experiment, a bioassay conducted in a peach orchard in Byron, Georgia (*8*), indicated that the most active materials were obtained by clipping the ovipositors of 1- to 3-day-old unmated female *S. pictipes* or *S. exitiosa* and extracting the ovipositors with methylene chloride or pentane, respectively (*9*). All subsequent steps in the

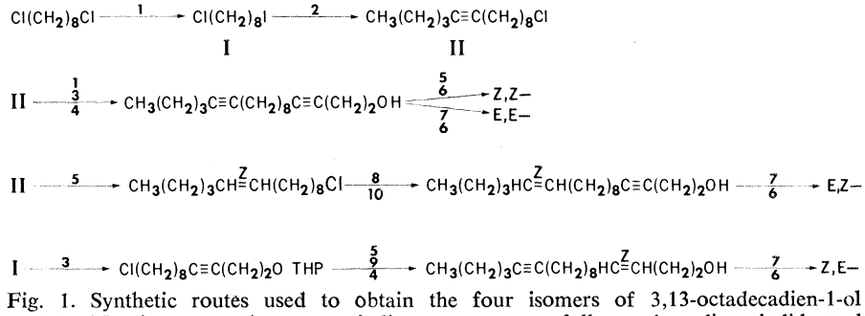

Fig. 1. Synthetic routes used to obtain the four isomers of 3,13-octadecadien-1-ol acetate. Numbers over the arrows indicate reagents as follows: 1, sodium iodide and acetone; 2, $CH_3(CH_2)_3C\equiv C^-Na^+$, liquid ammonia; 3, $THPO(CH_2)_3C\equiv C^-Na^+$, liquid ammonia (THPO, tetrahydropyranyl); 4, H^+, CH_3OH; 5, H_2, Pd; 6, acetic anhydride; 7, Na, liquid ammonia; 8, EDA · CH ≡ CH^-Li^+; 9, $CH_3(CH_2)_3-C\equiv C^-Li^+$; 10, ethylene oxide.