testis, anterior chamber of the eye, or to lymph nodes [in other studies (*9*)] may be due to a lack of hepatotropic factors in the blood perfusing these sites.

The portal vein route has previously been utilized successfully for transplantation of islets of Langerhans for the amelioration of diabetes in this and other laboratories (*10, 11*). Less islet tissue is required for cure when the portal vein route is used than when transplantation to other sites is performed. In addition, allogeneic transplantation has been performed with minimal immunosuppression, and it has been suggested that the portal vein and liver may be immunologically privileged sites (*11*). Data from studies on liver transplantation or from studies in which other organ allografts (heart, kidney) have been transplanted with venous drainage to the portal circulation support this hypothesis (*12, 13*). The mechanism of prolonged allograft survival is unknown, but it has been proposed that the liver inactivates or alters histocompatibility antigens (*13, 14*). In preliminary studies in our laboratory, however, hepatocyte transplantation via the portal vein without immunosuppression was not associated with lowering of recipient plasma bilirubin concentrations.

Bilirubin concentrations did not completely return to normal in any of the homozygous animals receiving intraportal hepatocytes, possibly because of the decreased enzyme levels in the hepatocytes obtained from the heterozygous animals used as donors, or perhaps because of insufficient immunosuppression. In our experiments, heterozygous Gunn rats were chosen as donors to minimize histocompatibility differences. In very similar experiments, performed recently and independently, Groth *et al.* (*15*) have observed a transient but unsustained fall in concentrations of bilirubin in nonimmunosuppressed homozygous Gunn rats infused via the portal vein with pure allogeneic hepatocytes (Wistar donors). Improved immunosuppression of homozygous recessive Gunn rats receiving a hepatocellular transplant from allogeneic donors with normal liver enzyme levels may result in lowering the concentration of plasma bilirubin of recipient animals even further.

Enzyme replacement therapy by cells capable of continuous enzyme production could be an effective mode of treatment of human enzyme deficiency diseases. The successful transplantation of UDPGT-synthesizing hepatocytes to Gunn rats lacking this enzyme provides a rational basis for such treatment. Technical application of this model would not be difficult. Only a small midline incision is necessary to isolate a loop of bowel and cannulate a mesenteric vein. Hepatocytes could be slowly infused and portal pressure measured before, during, and after infusion. The entire procedure could be performed with a local anesthetic. With current advances in prenatal diagnosis of congenital enzyme deficiency disease, it is possible to contemplate prenatal therapy. Enzyme-producing cells could be infused in utero or via the umbilical vein at birth. In utero transplantation could even result in tolerance to the transplanted cells, thus obviating the need for immunosuppression.

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Gnathotrichus sulcatus: Synergistic Response to Enantiomers of the Aggregation Pheromone Sulcatol

Abstract. *In laboratory and field bioassays,* Gnathotrichus sulcatus *responded to sulcatol (6-methyl-5-hepten-2-ol) only when both enantiomers were present. Response was greater to racemic sulcatol than to a mixture (65 : 35) of* S-(+) *and* R-(−) *enantiomers, the naturally occurring isomeric ratio. Enantiomer-specific active sites on receptor proteins in the same or different cells are implicated.*

The ambrosia beetle, *Gnathotrichus sulcatus* Le Conte (Coleoptera: Scolytidae) produces its aggregation pheromone, sulcatol (6-methyl-5-hepten-2-ol), in a mixture (65 : 35) of *S*-(+) and *R*-(−) enantiomers (*1*). We report that *G. sulcatus* responds to sulcatol only when both enantiomers are present, the first demonstration of synergistic response by insects to enantiomers of an attractive pheromone.

The ability of insects, particularly Lepidoptera, to distinguish between geometric isomers of volatile pheromones is well established (*2*). Only recently has research disclosed that insects are capable of olfactory discrimination between optical isomers. Oviposition by female spruce budworm moths, *Choristoneura fumiferana* (Clem.), is stimulated by exposure to *S*-(+)-α-pinene, but not to its *R*-(−) enantiomer (*3*). The ant, *Atta tex-*

Table 1. Response of *G. sulcatus* to traps baited with enantiomers of sulcatol (10 mg in 5 ml of benzene). Five replicates were made for each stimulus.

Stimulus	Caught (No.)	Sex ratio (F/M)
Experiment 1, 22 August to 3 September 1975		
Benzene (5 ml)	88a*	1.84
S-(+)-Sulcatol	182a	1.56
R-(−)-Sulcatol	68a	1.52
Mixture (65:35) of *S*-(+)- and *R*-(−)-sulcatol	1246b	1.60
(±)-Sulcatol	3021c	1.93
Experiment 2, 5 to 10 September 1975		
Benzene (5 ml)	18a	.38
(±)-Sulcatol	1009b	1.99
(±)-Sulcatol produced from synthetic *S*-(+) and *R*-(−) enantiomers	968b	1.93

*Values in each experiment followed by same letter are not significantly different by the Neuman Keuls test, $P < .05$. The data from experiment 1 were subjected to $\log_{10}(x + 1)$ transformation prior to analysis.

ana (Buckley), is 100 times more sensitive to the *S*-(+) enantiomer of its alarm pheromone, 4-methyl-3-heptanone, than to the *R*-(−) enantiomer (*4*). Synthetic (7*R*,8*S*)-(+) disparlure is more active than authentic racemic disparlure in laboratory bioassays and electroantennogram recordings, whereas the (7*S*,-8*R*)-(−) enantiomer is only marginally active (*5*). Among the Scolytidae, the western pine beetle, *Dendroctonus brevicomis* Le Conte, can distinguish between the enantiomers of its aggregation pheromones brevicomin and frontalin (*6*), the Douglas-fir beetle, *D. pseudotsugae* Hopkins, is more sensitive to *R*-(−)- than to *S*-(+)-frontalin (*7*), and the southern pine engraver, *Ips grandicollis* (Eichhoff), aggregates only in response to the *S*-(−) isomer of ipsenol (*8*). The development of syntheses for the enantiomers of sulcatol (*9*) allowed us to test the response of *G. sulcatus* to the optical isomers of its aggregation pheromone.

The enantiomers of sulcatol were synthesized from the enantiomers of glutamic acid as described by Mori (*9*). Racemic sulcatol was synthesized by the sodium borohydride reduction of 6-methyl-5-hepten-2-one (*1*). For laboratory and field bioassays, the pheromone was diluted in benzene.

Field bioassays were conducted in a selectively logged forest at Point Roberts, Washington. Pheromones were deployed in a vial within an inverted vial release system in cylindrical wire mesh traps (0.3 m^2) coated with Stickem Special (*1*). The first field experiment ran for 12 days, and comprised a 5 by 5 Latin square design with the following stimuli: benzene solvent controls, *S*-(+)-sulcatol, *R*-(−)-sulcatol, (±)-sulcatol, and a mixture (65 : 35) of *S*-(+) and R-(−) enantiomers [formed by adding synthetic *S*-(+)-sulcatol to a racemic mixture]. All pheromone stimuli were at a concentration of 10 mg in 5 ml of benzene. Traps were deployed along logging spur roads, at a minimum between-trap spacing of 30 m. An average pheromone release rate of 4 to 8 μg/hour was determined by weighing, after the experiment, the residual pheromone in the bait vials.

Response of naturally occurring *G. sulcatus* disclosed an overwhelming preference for racemic sulcatol (Table 1, experiment 1), significantly greater than for all other stimuli. The 65 : 35 mixture of *S*-(+) and *R*-(−) enantiomers was at least seven times more attractive than either isomer alone, neither of which was significantly more attractive than the solvent controls. The sex ratio did not vary significantly with treatment, indicating that there is no sexual difference in chiral receptor systems.

Laboratory bioassays were performed with walking beetles in an open-stage, arena type olfactometer (*10*). Only females were tested, since the response of males in this bioassay is minimal (*1*). To preclude observer error, the identity of stimuli was not disclosed to the investigator until an entire bioassay series was complete.

The laboratory bioassay response produced results comparable with those in field tests (Table 2). Response to the racemic mixture was greater than to the 65 : 35 mixture of *S*-(+) and *R*-(−) enantiomers, which in turn was significantly greater than that to either of the two enantiomers tested alone. Neither enantiomer induced a response greater than that to the solvent controls.

The responses to (±)-sulcatol and the 65 : 35 mixture of *S*-(+) and *R*-(−)-sulcatol (Tables 1 and 2) suggested synergism between the optical isomers of sulcatol. However, the unlikely hypothesis remained that the synthetic enantiomers were accompanied by minute quantities of a pheromone-masking, synthesis byproduct. This hypothesis was tested by adding the two synthetic enantiomers together in equal amounts, and assaying this racemic mixture against the racemic mixture produced by sodium borohydride reduction of 6-methyl-5-hepten-2-one. In laboratory bioassays, and a second field experiment (single-factor design with five replicates), both racemic mixtures were competitive with each other (Tables 1 and 2), thus eliminating all possibilities of pheromone masks (or inactivity) in the synthetic enantiomers. However, we have no explanation for the significantly lower response to the 65 : 35 mixture of *S*-(+) and *R*-(−) enantiomers, the form in which sulcatol is synthesized by male *G. sulcatus* (*1*).

Table 2. Response of female *G. sulcatus* to enantiomers of sulcatol (1 μg in 0.05 ml of benzene) in a laboratory olfactometer.

Stimulus	Tested (No.)	Responders (No.)	Response (%)
Benzene (0.05 ml)	101	4	3.6a*
S-(+)-Sulcatol	100	7	7a
R-(−)-Sulcatol	100	6	6a
65:35 mixture of *S*-(+)- and *R*-(−)-sulcatol	98	19	19.4b
(±)-Sulcatol	98	42	42.9c
(±)-Sulcatol produced from synthetic *S*-(+) and *R*-(−) enatiomers	101	42	41.6c

*Values followed by the same letter not significantly different; χ^2 test, $P < .05$.

There are at least two possible means by which the synergistic response to optical isomers of a pheromone could be mediated. The first mechanism would involve enantiomer-specific receptor cells, either on separate sensilla, or within the same sensillum. A behavioral response would occur only when both receptors were excited and would follow central nervous system integration of the two signals. The second perception mechanism might involve enantiomer-specific active sites on the receptor protein or proteins of the same receptor cell. Although there are no data on optical isomers, there is evidence that geometric isomers can be distinguished by the same receptor cell. For example, one type of receptor cell associated with the sensilla trichodea of the red-banded leaf roller, *Argyrotaenia velutinana* Walker, responds in an excitatory manner to the sex pheromone, *cis*-11-tetradecenyl acetate, but displays slight inhibitory responses to the *trans* isomer (*11*). A synergistic system involving more than one receptor site on the same receptor protein might be similar to certain enzyme substrate systems. For example, tryptophan oxygenase activity may be regulated such that exposure of an allosteric site to tryptophan or α-methyltryptophan will ensure that the normal catalytic activity of the enzyme is preserved (*12*).

We have no evidence for the natural significance of a synergistic response to pheromone enantiomers, or of enantiomer-specific responses. However, such phenomena could provide means of ensuring reproductive isolation between species utilizing either pheromone enantiomer, or different enantiomeric ratios. Alternatively, as also proposed by Renwick *et al.* (*13*), the host range of an insect might be limited to those host species, races, or individuals that offer pheromone precursors of appropriate enantiomeric composition.

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Western Pine Beetle: Specificity Among Enantiomers of Male and Female Components of an Attractant Pheromone

Abstract. *The flight response of both sexes of* Dendroctonus brevicomis *to the mixture of myrcene, racemic frontalin, and (1*R,5S,7R*)-(+)-*exo*-brevicomin and to the mixture of myrcene, (1*S,5R*)-(−)-frontalin and racemic* exo*-brevicomin was significantly greater than the response to the same mixtures in which the antipodes were substituted. The flight response to these two mixtures was also greater than the response to the ternary mixture of myrcene, racemic frontalin, and racemic* exo*-brevicomin (MFE). The walking response of both sexes to the mixture of myrcene, racemic frontalin, and (+)-*exo*-brevicomin was not different from the response to MFE. Substitution of the antipode lowered the response when compared to that of MFE. When evaporated with ponderosa pine turpentine, (−)-frontalin was active in the field while its antipode was not.*

We have discovered that (1*R*,5*S*,7*R*)-(+)-*exo*-brevicomin and (1*S*,5*R*)-(−)-frontalin are active in the ternary mixtures containing the host-produced component, myrcene. The mixtures containing these compounds elicited both a walking and flight response from male and female *Dendroctonus brevicomis* Coleoptera: Scolytidae), while mixtures containing their antipodes were much less attractive.

Exo-Brevicomin (*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane) and myrcene, a component in the oleoresin of ponderosa pine, were isolated from frass produced by females boring in ponderosa pine (*1*, *2*), and frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) was isolated from hindguts of males (*3*). Subsequently, the combination of these two bicyclic ketal pheromones with ponderosa pine oleoresin and turpentine (*4*, *5*) and with myrcene (*6*) were shown to be highly attractive to both sexes in the field. Further, both of these ternary mixtures were shown to be much more attractive than any binary mixtures containing these or other compounds. Single compounds were either not attractive or they were much less attractive than the binary mixtures. Because these differences in response levels were not additive, the term synergism was used to describe this phenomenon. These evaluations (*5*, *6*) were made with racemic frontalin and *exo*-brevicomin. Recently Mori has synthesized the enantiomers of *exo*-brevicomin (*7*) and frontalin (*8*). Silverstein and co-workers (*9*) found (1*R*,5*S*,7*R*)-(+)-*exo*-brevicomin in cold-trap condensates from aerated ponderosa pine logs infested with females alone or with females and males. They also found (1*S*,5*R*)-(−)-frontalin in extracts of the hindguts of males (*9*). As a result of these studies, we sought to determine the attractant or interruptant activity of these optically active compounds (or both). The following notation is used throughout this report: Racemic *exo*-brevicomin (E), racemic frontalin (F), myrcene (M); F or E preceded by (+) or (−) refers to the enantiomer, and (±) refers to a mixture of both enantiomers in equal proportions.

In laboratory studies, the attractiveness of the enantiomers in various mixtures was evaluated in an open-arena olfactometer (*10*). A power-driven microsyringe was utilized to deliver the compounds (*11*). Each compound was dissolved in pentane (1 ng/μl) and delivered at the rate of 2 μl/min; a pentane check was also delivered at 2 μl/min. Beetles were released in groups of five or ten, and a positive response was recorded when an individual had traversed the 20 cm upwind to the attractant source.

Field tests were conducted at Grass Valley (Nevada County) and Bass Lake (Madera County), California. Both locations are at an elevation of approximate-

Table 1. Walking response of *D. brevicomis* to the combinations of *exo*-brevicomin (E) and frontalin (F) enantiomers, racemates, and myrcene (M). The data were obtained 4 to 11 June 1974.

Treatment	Males			Females		
	Number tested	Proportion responding	Confidence interval*	Number tested	Proportion responding	Confidence interval
Pentane	30	.066	.02–.23	10		
M,F	20	.10	.02–.32			
M,F,E	20	.65	.41–.84	10	.80	.44–.97
M,F,(±)E	20	.65	.41–.84	10	.50	.18–.81
M,F,(+)E	30	.50	.29–.78	20	.80	.56–.93
M,F,(−)E	30	.166	.06–.35	20	.15	.03–.37

*Confidence limits were estimated from a chart of binomial confidence limits (95 percent) for proportions. Differences between two proportions responding are highly significant if the intervals of the two proportions do not overlap.