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Chirality of Ipsdienol and Ipsenol Indicates a Frass Pheromone System in the Spruce Engraver, *Ips typographus*

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The spruce engraver, *Ips typographus* L. (Col., Scolytidae), produces and responds to (*R*)-(−)-ipsdienol and (*R*)-(+)-ipsenol as additional compounds in the beetle's aggregation pheromone. In contrast, other *Ips* spp. so far known to employ optically pure ipsdienol and ipsenol use the combination (*S*)-(+)-ipsdienol and (*S*)-(−)-ipsenol as aggregation signal.

At epidemic population levels, *I. typographus* successfully attacks Norway spruce, *Picea abies* (Karst.) L., by aggregation *en masse,* causing severe economic losses in mature spruce stands [1 – 4]. Mass aggregation on spruce trees is achieved primarily by the aggregation pheromone consisting of the terpene alcohol (*S*)-cis-verbenol and the hydroxylated isoprene derivative 2-methyl-3-buten-2-ol (MB), acting as obligatory synergists [5 – 7] during landing and gallery initiation [8,9]. After successful construction of the mating chamber, however, male *I. typographus* release minor amounts of the terpene alcohols ipsdienol and ipsenol [9 – 12], semiochemicals nearly ubiquitous among the genus *Ips* DeGeer [13].

We used the technique of juvenile hormone treatment [14] to enhance ipsdienol and ipsenol production in *I. typographus.* Juvenile hormone JH 3 [15] diluted in acetone was topically applied to beetles collected from traps in the field. Subsequently, the beetles were placed in the dark and exposed for ca. 24 h to vapors of technical myrcene (>78 %, Aldrich), racemic (rac.) ipsdienol (>95 %, Borregaard), and ipsdienone (>84 %), respectively. After exposure, beetles were immobilized on dry ice, dissected, and excised hindguts stored in pentane at −80 °C until further analysis. In addition, male *I. typographus* were removed from a spruce trap tree with advanced galleries containing eggs [10 – 12]. Enantiomeric determination of ipsdienol and ipsenol was performed by the use of a fused silica column (30 m long, 0.25 mm i.d.) coated with peracetyl α-cyclodextrin operated under a temperature program of 50 to 150 °C at a rate of 1 °C/min. Assignment of absolute configurations of natural compounds is based on comparison of retention times of synthetic, optically active reference samples.

Field tests were carried out in mixed spruce stands in the Black Forest at Buchenbach near Freiburg/Br. (FRG) using black "Flachtricher" (Röchling) flight barrier traps set up in pairs or triangles with approx. 10 – 15 m between traps. Traps were baited with synthetic (*S*)-cis-verbenol (>95 %, ee = 92 %, Borregaard), rac. ipsenol (> 85 %, Borregard), (R)-(+)-ipsenol (> 94 %, ee > 99 %), (*S*)-(−)-ipsenol (>93 %, ee>99 %), MB (>97 %, Fluka), and 2-methyl-3-butyn-2-ol (MBi; >98 %, Merck-Schuchardt), respectively. The test compounds evaporated from sponge tissue sealed in lowdensity (50 μm) polyethylene bags (7 × 10 cm), glass vials (50 × 9 mm) covered with a perforated plastic stopper, and/or soda-glass capillaries (4.5 cm long, 1.2 mm i.d.) sealed at one end and placed in glass vials with a perforated stopper. To avoid contamination and minimize positional effects, traps including the bait material were systematically interchanged within pairs or triangles after every control. The sex ratio of the beetles caught was obtained by dissection.

Upon prolonged feeding in the trap tree, *I. typographus* males produced (*R*)-(−)-ipsdienol and (*R*)-(+)-ipsenol (ratio ca. 2:1). This result corroborates earlier findings showing *I. typographus* to produce (*R*)-(−)-ipsdienol [16] and to respond preferentially to this enantiomer [17]. The antipode, (*S*)-(+)-ipsdienol, did not substantially affect the number of beetles caught [17,18]. Like other *Ips, Pityokteines,* and *Dendroctonus* spp. [13,14,19], *I. typographus* males appear capable of converting the achiral host-specific monoterpene myrcene into chiral ipsdienol and ipsenol (Table 1). Females, if ever, contain only traces of ipsdienol/ipsenol after exposure to myrcene vapors. However, due to possible mistakes during sex determination upon dissection, the batch samples of *I. typographus* females might have included some male hindguts. Also, the exposure of *I. typographus* males to rac. ipsdienol resulted in the production of trace amounts of ipsenol. It is interesting to note that exposure to myrcene following JH treatment yielded ipsenol in considerably lower optical purities as compared to the compound produced during feeding in host tissue under natural conditions (Table 1). Sex-specific differences became obvious in the reduction of ipsdienone: males reduce the ketone more effectively to the terpene alcohols than females. Again, the optical purity of the reduction prod-

Table 1. Content and chirality of ipsdienol and ipsenol in hindguts of *I. typographus* males removed from a trap tree or exposed to vapors of different synthetics. X: Identity confirmed by gc-ms; amount too small for determination of the enantiomeric composition

Beetle treatment	Number of beetles analyzed	Ipsdienol	Ipsenol
Trap tree	142	(*R*)-(−) ee = 90 %	(*R*)-(+) ee = 90 %
Myrcene-exposed	628	X	(*R*)-(+) ee = 10 %
Ipsdienone-exposed	189	(*R*)-(−) ee = 60 %	X

Table 2. Field response of *I. typographus* to flight barrier traps baited with attractants with or without addition of (*R*)-(+)-ipsenol (1990; I: 2 trap pairs, 14 controls, July 26 – August 16, II: 2 trap pairs, 12 controls, August 2 – 16)

Trap bait	*I. typographus* caught $\bar{x} \pm SE$ (♂ : ♀)
I (27 replicates)	
A: MB[a], (*S*)-cis-verbenol[b]	30.9 ± 4.55
B: A plus (*R*)-(+)-ipsenol[c]	31.8 ± 6.64
II (23 replicates)	
C: A plus MBi[d]	17.3 ± 2.82* (1:4.7)
D: A plus (*R*)-(+)-ipsenol in MBi[e]	21.6 ± 4.23* (1:4.4)

[a]2 ml, polyethylene bag. [b]50 mg, glass vial. [c]10 µl, 1 capillary. [d]1 ml, polyethylene bag. [e]20 µl diluted in 1 ml, polyethylene bag.
* Differences between means significant for $p < 0.19$ (2-tailed sign test) or $p < 0.41$ (Wilcoxon matched pairs signed rank test).

uct was found to be lower than that of the "naturally" produced ipsdienol.

I. paraconfusus (Lanier) [20], *I. cembrae* (Heer) [21], and *I. acuminatus* (Gyll.) [22,23] use (*S*)-(+)-ipsdienol/(*S*)-(−)-ipsenol as aggregation signals; *I. typographus* produces just the opposite enantiomers. These findings are supported by earlier experiments with *I. typographus* employing ipsdienol of varying enantiomeric composition: exposure to vapors of (*S*)-(+)-ipsdienol yielded ipsenol in amounts easily detectable by gc in male hindguts while exposure to rac. ipsdienol resulted in trace amounts of ipsenol only (J. P. Vité, unpubl. results). The field response of *I. typographus* to ipsenol enantiomers observed in the Black Forest (Tables 2, 3) corroborates earlier findings reported from Norway [24]. The addition of (*S*)-(−)-ipsenol clearly reduces beetle response while (*R*)-(+)-ipsenol seems to slightly enhance trap catches although differences were not statistically significant. Apparently, the strong response inhibition reported earlier for rac. ipsenol [24,25] rests with the enantiomer non-natural to *I. typographus*. Hence, recent speculations on the possible role of ipsenol in density regulation [25] are likely to base on misinterpretation of results obtained with rac. ipsenol. As predicted by Bakke [24] "... an explanation of the actual biological function of ipsenol may rest until the absolute configuration of ipsenol released by *I. typographus* has been established." *I. paraconfusus,* and probably *I. cembrae* as well, are capable of stereoselectively converting (*R*)-(−)-ipsdienol to (*S*)-(+)-ipsenol [26]. Our findings suggest the opposite stereoselectivity for the corresponding transformation in *I. typographus*.

Our data support the concept of "contact" and "frass" pheromones [27,28] and their significance in an evolutionary context: terpene alcohols derived from achiral myrcene as frass pheromones are common to most, if not all, *Ips* spp. Speciation within the genus is accompanied with splitting this group of semiochemicals into specific aggregation signals by combination, release rate, and/or absolute configuration of compounds. While frass pheromones ensure the colonization of temporary habitats, mass aggregation on resistant host trees by aggressive species requires contact pheromones. We, therefore, present the hypothesis that in *I. typographus* aggressiveness secondarily evolved with (*S*)-cis-verbenol and MB as contact pheromones, while the natural enantiomers of the ipsdienol/ipsenol pair still function as the original species-specific frass pheromones [29]. It remains to be clarified whether the traces of the ipsdienol/ipsenol pair are an evolutionary carryover or, perhaps, actually present an independent alternative frass pheromone in competitive colonization of temporary habitats not allowing for satisfactory production of the contact pheromone (*S*)-cis-verbenol [7,8,12,30]. However, to verify this hypothesis further tests with optically active compounds are needed.

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Table 3. Field response of *I. typographus* to flight barrier traps baited with attractants with or without addition of rac. or (*S*)-(−)-ipsenol (1989; I: 3 trap pairs, 6 controls, June 16 – 20; II: 3 trap pairs, 3 controls, June 23 – 28; III: 2 trap triangles, 4 controls, May 5 – 20)

Trap bait	*I. typographus* caught $\bar{x} \pm SE$
I (18 replicates)	
A: (*S*)-cis-verbenol, MB[a]	149.1 ± 22.04 A
B: A plus (*S*)-(−)-ipsenol[b]	69.4 ± 13.52 B
II (9 replicates)	
C: A plus MB[c]	109.0 ± 21.35 C
D: A plus (*S*)-(−)-ipsenol in MB[d]	36.9 ± 8.54 D
III (8 replicates)	
E: A plus MBi[e]	20.6 ± 4.37 E
F: A plus rac. ipsenol in MBi[f]	11.6 ± 3.68 E, F
G: A plus (*S*)-(−)-ipsenol in MBi[f]	9.1 ± 3.06 F

[a]Verbenol diluted in MB (1:4), 2 capillaries, each loaded with 20 µl. [b]0.2 ml, glass vial. [c]20 µl, 1 capillary. [d]4 µl in 16 µl, 1 capillary. [e]2 ml, polyethylene bag. [f]40 µl in 2 ml, polyethylene bag.
A – D: means of pairs differ significantly if followed by different letters ($p < 0.01$, Mann-Whitney *U*-test).
E, F: differences between treatments significant at $p < 0.08$ (Kruskal-Wallis oneway analysis of variance). Differences between means significant at $p < 0.02$ (Mann-Whitney *U*-test) are followed by different letters.

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Neurodepressing Effect of Brassinosteroids in the Cockroach *Periplaneta americana*

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Brassinosteroids have striking similarities in their chemical structure to ecdysteroids (Fig. 1). They are only known to occur in plants, whereas ecdysteroids are distributed in plants as well as in all phyla of protostomian animals. Brassinosteroids are potent growth-promoting regulators in plants, but on the other hand, there is no striking evidence for effects of ecdysteroids in plants [1]. 20-OH-ecdysone is the molting hormone in arthropods [2]. Besides their well-known influence on gene expression in ectodermal epithelia of arthropods, ecdysteroids are shown in some instances to have neurotropic effects involved in the molting of insects [3]. The spike activity of the nervus corporis cardiaci II (Ncc II), connecting the insect brain with the retrocerebral neurohormonal/glandular complex, is inhibited by 20-OH-ecdysone as a feedback mechanism [4]. The chemical relationship to ecdysteroids is one reason to investigate the effects of brassinosteroids in insects. Another reason is the potential significance of brassinosteroids in plant cultivation. Their application to plants includes various possibilities of contacts with insects. This may lead to interferences of ecdysteroid-regulated processes with brassinosteroids.

Experiments with application of brassinosteroids on insects have shown a molt-delaying effect in *Periplaneta americana* [5] and antiecdysone effects by competition on the ecdysteroid binding sites in nuclear fractions of blowfly larvae (*Calliphora vicina*) [6] and in the

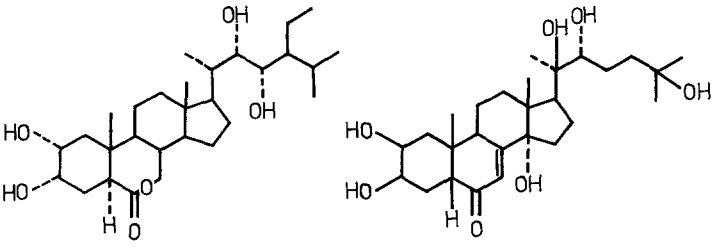

Fig. 1. Structural formulae of the brassinolide and the ecdysteroid type of steroids