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PHEROMONE BIOSYNTHETIC PATHWAYS: CONVERSIONS OF DEUTERIUM LABELLED IPSDIENOL WITH SEXUAL AND ENANTIOSELECTIVITY IN IPS PARACONFUSUS LANIER

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The biosynthesis of insect pheromones is an extremely exciting area of much recent interest[1-9]. The studies involved with *Ips paraconfusus* Lanier have been concerned with the *in vivo* conversion of host terpene hydrocarbons, such as α-pinene and myrcene, to pheromones of this species. Thus, Renwick *et al.*[5] showed that volatilized (-) α-pinene was converted to (+) *cis*-verbenol, while Brand *et al.*[7] provided evidence that the biosynthetic conversion with (±) α-pinene to *cis*- and *trans*-verbenol is related to certain bacteria isolated from the gut of adult male and female bark beetles. Hughes[3] provided tentative evidence that myrcene was converted to ipsdienol and ipsenol upon topical application of this precursor terpene hydrocarbon. In this latter study, as far as we can judge, only gas-liquid chromatography (GC) retention times and no other criteria were used to ascertain this biosynthetic route. More recently, we have determined, utilizing GC in conjunction with chemical ionization mass spectrometry (GC-CIMS), as well as bioassays, that myrcene is indeed converted sex specifically (males) to ipsdienol and that ipsenol is also formed[10].

In this communication, we wish to demonstrate, in an unequivocal fashion by using deuterium labelling techniques, that ipsdienol is the biosynthetic precursor to ipsenol, and that this biotransformation is accomplished by males only with the (-) enantiomer. More importantly, we discovered that not only is ipsdienol-d converted to ipsenol-d, but that ipsdienone, the ketone analog of ipsdienol, is also a precursor to ipsenol.

The racemic ipsdienol-d was prepared in the following manner. Ipsdienol was oxidized using pyridinium chlorochromate in the presence of sodium acetate to ipsdienone (61% yield)[11]. The ipsdienone was then converted to ipsdienol-d by reaction with sodium borodeuteride in isopropyl

alcohol[12]. Preparative GC (4.5 m x 10 mm carbowax 20M, glass column) gave pure material as analyzed by ^{1}H nmr (90 MHz Perkin Elmer R32 in the Fourier Transform mode) and GC-CIMS (Finnigan 1015D, isobutane, 4.5 m x 10 mm OV101 at 100°). CIMS analysis showed the m/e 135 and 136 ions $[M-17]^+$ providing a 64% deuterium incorporation in the product.

When the male beetle penetrates the bark of its host, it is exposed to tree-produced oleoresins through ingestion, aeration and contact. Accordingly, beetles were exposed to volatilized precursors by aeration (24 hr). Groups of 100 or less bark beetles were confined separately by sex in 400 ml dark glass bottles along with silane treated glass wool, which was added to separate the beetles from each other and from the liquid precursor (10 to 50 µl of precursor spotted on small pieces of glass filter paper).

In preliminary experiments, using these techniques, it was shown that males only were responsible for the conversion of ipsdienol to ipsenol. A similar experiment was conducted using ipsdienol-d and after 24 h the pheromones were isolated by pulling the abdomen from the thorax and extracting it with diethyl ether. The ipsdienol-d and ipsenol-d (Table 1) were purified by preparative GC (4.5 m x 10 mm carbowax 20M at 100°C) and then analyzed by GC-CIMS (isobutane reagent gas), m/e 136/135 $[M-17]^+$, 59% deuterium [ipsdienol-d]) and m/e 138/137 $[M-17]^+$, 25% deuterium [ipsenol-d]) and 180 MHz ^{1}H FT nmr ([C_6H_6-d_6/TMS], 1.47, 1.56, 2.40, 4.52, 4.91, 4.97, 5.18, 5.20, 5.26, 6.27 ppm [ipsdienol-d], and 0.85, 0.93, 1.36, 1.83, 2.27, 3.77, 4.95, 5.25, 6.31 ppm [ipsenol-d]) to provide unequivocal evidence for their assigned structures. The GC-CIMS results clearly reveal deuterium incorporation in the ipsenol-d formed in the biosynthesis; however, the deuterium content was found to be reduced from 64% d to 25% d.

Table 1: GC-CIMS Verification and GC Quantification of Pheromone Conversions by *I. paraconfusus* following a 24 h Exposure to Volatized Precursors.[1]

Number and Sex of Beetles Exposed	Volatized Precursors	Conversion Products Detected in Abdomens (micrograms/abdomen).		
320 ♂♂	ipsdienol-d	ipsenol-d	0.7-1.5	
82 ♀♀	ipsdienol-d	ipsenol not detected[2]		
100 ♂♂	(-) ipsdienol	ipsenol	1.1	
100 ♂♂	(+) ipsdienol	ipsenol	.14-.34	
50 ♀♀	(-) ipsdienol	ipsenol	not detected	
50 ♀♀	(+) ipsdienol	ipsenol	not detected	
50 ♂♂	ipsdienone	ipsenol	0.26	ipsdienol 0.02
50 ♀♀	ipsdienone	ipsenol and ipsdienol	not detected[3]	
50 ♂♂	air only	ipsenol and ipsdienol	not detected[3]	

1) 4.5 m x 4 mm Carbowax 20M Column (GC) and OV101 Column (GC-CIMS)
2) GC maximum sensitivity with this extract was 0.1 µg/abdomen.
3) GC maximum sensitivity with this extract was 0.002 µg/abdomen.

One plausible explanation for the reduced deuterium content was that of a separate pathway to ipsenol by further oxidation of the ipsdienol-d to ipsdienone, which would account for a los of deuterium at the carbon atom bearing the hydroxy group. The latter could then be reconverted to unlabelled ipsdienol and then by reduction of the double bond to unlabelled ipsenol.

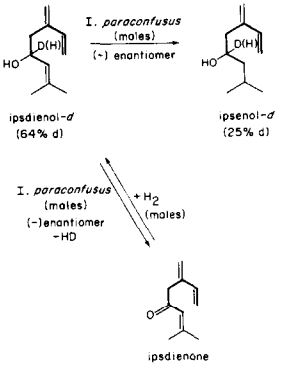
We verified this pathway by aeration of the male and female bark beetles with the ketone and found that males converted ipsdienone to ipsdienol and ipsenol (Table 1). The observations of (1) partial deuterium loss in the *in vivo* conversion of ipsdienol-d (64%) to ipsenol-d (25%); (2) the *in vivo* conversion of ipsdienone to both ipsdienol and ipsenol; (3) the loss of deuterium in the recovered ipsdienol (64% d to 59% d) strongly support dual biosynthetic pathways to ipsenol from ipsdienol. Furthermore, we are conducting experiments to define the role of the ketone analog of ipsenol, ipsenone, to see if it is formed from either ipsdienone and/or ipsenol.

Enantiomeric specificity has recently been shown to be important for the maximum biological activity of insect pheromones[13-16]; therefore, we aerated male and female beetles with the (+) and (-) enantiomers of ipsdienol[17]. The (-) enantiomer (90% e.e., enantiomeric excess, as determined by GC using R - (+) - *trans*-chrysanthemic acid as the resolving agent) was converted by males to ipsenol as analyzed by GC-CIMS, while the (+) enantiomer (74% e.e.) gave only small amounts (four to eight times less than the (-) enantiomer) of ipsenol as expected due to ~13% of the (-) enantiomer being present in the (+) ipsdienol. The female bark beetles apparently converted neither the (+) nor (-) enantiomers of ipsdienol to ipsenol. Consequently, these results allow formulation of several plausible biosynthetic pathways from ipsdienol to ipsenol (Scheme).

Scheme:

Biosynthetic pathways from

Ipsdienol to Ipsenol.



This report constitutes the first definitive study in which one insect pheromone component is used as a biosynthetic precursor to another pheromone and clearly demonstrates the high specificity, both sexual and sterochemical, that occurs in these chemical communication systems. It

also illustrates the distinct advantages of specific deuterium labelling techniques in elucidating pheromone biosynthetic pathways.

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