1 Present address: Friday Harbor Marine Laboratory, 620 University Road, Friday Harbor, WA 98 250 (USA).
2 Faulkner, D. J., and Ghiselin, M. T., Mar. Ecol. Prog. Ser. *13* (1983) 295; Faulkner, D., J. nat. Prod. Rep. *1* (1984) 251; Carefoot, T. H., Mar. Biol. Ann. Rev. *25* (1987) 167.
3 Norris, J. N., and Fenical, W., Smithsonian Contrib. Mar. Sci. *12* (1982) 417.
4 Hay, M. E., and Fenical, W., A. Rev. Ecol. Syst. *19* (1988) 111. Paul, V. J., and Van Alstyne, K. L., J. exp. mar. Biol. Ecol. *119* (1988) 15.
5 Hay, M. E., Pawlik, J. R., Duffy, J. E., and Fenical, W., Oecologia (1989) Submitted for publication.
6 Pawlik, J. R., Albizati, K. F., and Faulkner, D. J., Mar. Ecol. Prog. Ser. *30* (1986) 251.
7 Compound **1** ^{1}H NMR (360 MHz in C_6D_6). 6.48, (1H, s, H-5), 6.41, (1H, s, H-8), 5.38, (1H, t, J = 6.6 Hz, H-3), 3.12, (3H, s, H-15), 2.12, (3H, s, H-14), 1.96, (2H, m, H-2), 1.67, (3H, s, H-12), 1.60, (3H, s, H-13), .90, (3H, t, J = 7.5 Hz, H-1). ^{13}C NMR (50 MHz) 180.5 (s), 162.1 (s), 159, 0 (s), 136.0 (d), 134.7 (d), 131.5 (s), 125.6 (s), 110.5 (d), 101.1 (s), 54.7 (q), 21.9, 16.5 (t), 13.9 (q), 13.9 (q), 7.1 (q). IR (cm^{-1}, film): 2960, 2920, 1650, 1610, 1460, 1315, 1260, 1170.
8 Compound **2** ^{1}H NMR (360 MHz). 5.96, (1H, s, H-5), 5.51, (1H, s, H-10), 5.33, (1H, t, J = 7.4 Hz, H-3), 2.91, (3H, s, H-15), 2.12, (3H, s, H-14), 1.91, (2H, m, H-2), 1.84, (3H, s, H-12), 1.54, (3H, s, H-13), .86, (3H, t, J = 7.5 Hz, H-1), ^{13}CMR (50 MHz, data incomplete due to limited amount of compound) 167.3 (s), 139.4 (d), 135.6 (d), 125.9 (s), 88.4 (d), 55.0 (q), 21.7 (q), 16.2 (t), 16.1 (q), 13.9 (q), 11.7 (q). IR (cm^{-1}, film): 2960, 2920, 1740, 1640, 1570, 1450, 1420, 1380.
9 Hochlowski, J. E., and Faulkner, D. J., Tetrahedron Lett. *1917* (1983); Turner, W. V., and Pirkle, W. H., J. org. Chem. *39* (1974) 1935; Pelter, A., and Ayoub, M. T., J. chem. Soc. Perkin I *1174* (1981); Poulton, G. A., Cyr, T. D., and McMullan, E. E., Can. J. Chem. *57* (1979) 1451.

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Conversion of phenylalanine to toluene and 2-phenylethanol by the pine engraver *Ips pini* (Say) (Coleoptera, Scolytidae)

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Summary. The pine engraver, *Ips pini* (Say), was found to produce toluene and 2-phenylethanol when boring into fresh pine logs. The hypotheses that phenylalanine is a precursor of these compounds and that beetles without their symbiotic microorganisms can perform these conversions were confirmed by treating wild and axencially-reared males and females topically with L-phenyl-d_5-alanine. Extracts of these beetles invariably contained deuterio-toluene, and extracts of males contained deuterio-2-phenylethanol as well.
Key words. Insecta; Scolytidae; phenylalanine; aromatics; axenic.

Phenylalanine plays a central role in insect physiology, acting as an essential nutrient[5], a major constituent of cuticle[6], and a component of structural and storage proteins[7,8]. It has also been shown to be behaviourally active, stimulating host-searching in the parasitoid *Apanteles cypris*[9], feeding behaviour in *Lygus lineolaris*[10], and positive chemotaxis in larval *Culex pipiens quinquefasciatus*[11]. Moreover, phenylalanine serves as the precursor for *p*-benzoquinone production in the defensive secretions of *Elodes longicollis*[12].

Despite the varied fates of this amino acid, only two modes of phenylalanine metabolism have been described in insects. Phenylalanine can be hydroxylated to tyrosine prior to incorporation into proteins, a common metabolic pathway. In a far less common reaction, males of the bertha armyworm, *Mamestra configurata*, convert phenylalanine into the pheromone 2-phenylethanol[13].

Volatiles captured from individual gallery systems of the pine engraver, *Ips pini*[14], contained toluene and 2-phenylethanol, aromatic volatiles without ring hydroxylation. Since the mean hourly release of 226 ng of toluene (SD = ± 195, n = 18) during 30 h of aeration could not be explained as atmospheric contamination, and insects do not produce aromatic structures themselves but receive them from their diets, we tested the hypothesis that phenylalanine is a precursor of the toluene and 2-phenylethanol found in *I. pini* volatiles. Since microbial symbionts can be involved in pheromone biochemistry and volatile production in Scolytids[15–18], and *Ips pini* carries several species of blue stain fungi and yeasts[19,20], we also tested whether the beetles can perform these conversions of phenylalanine without their symbiotic microorganisms.

Materials and methods

Experimental insects. Experiments were conducted on wild and axenically-reared male and female *I. pini*. Axenic beetles lacking culturable microorganisms were reared as previously described for *Dendroctonus ponderosae*[21], and were allowed to feed and mature for 2–6 weeks as adults. Wild beetles were allowed to emerge from logs of naturally infested lodgepole pine, *Pinus contorta* var. *latifolia*, held in screened cages at approximately 26 °C on a photocycle of 16:8 (L:D). Beetles were collected daily, separated by sex, grouped into 8–14 individuals per replicate, and used that day.

Phenylalanine treatment. 20 mg of L-phenyl-d_5-alanine labelled exclusively in the phenyl ring (lot No. 2250-L, Merck Frosst Canada Inc., Montreal) were dissolved in

2.0 ml of double distilled water/residue grade acetone (1:1); 0.5 µl of this solution, containing 5 µg of phenylalanine, was applied to the ventral surface of each beetle. Following evaporation of the solvent, the beetles were placed in layers of filter paper in glass jars. Axenically-reared beetles were treated similarly under aseptic conditions in a laminar air flow hood. After 18 h of storage at room temperature, beetles treated with phenyl-d_5-alanine as well as untreated and solvent-treated control beetles were crushed for 2 min in redistilled pentane/ether (95/5) over dry ice. Beetle extracts and the solution used for topical treatment were subjected to analysis by coupled gas chromatography-mass spectrometry (GC-MS), employing a Hewlett-Packard 5985B GC-MS fitted with a 30 m × 0.32 mm ID DB-1 column. Protio- and deuterio-toluene were quantified, whereas the presence of 2-phenylethanol was only monitored because amounts were too small for reliable quantification. Data were transformed by $\log(\times + 1)$ to remove heteroscedasticity (Bartlett-Box, $F = 148$ and $F = 0.3$, $p = 0.22$ and $p = 0.83$ for protio-toluene and deuterio-toluene, respectively), and group means were compared by the Student Newman Keuls test using SPSSPC statistical software (SPSS Inc., Chicago IL).

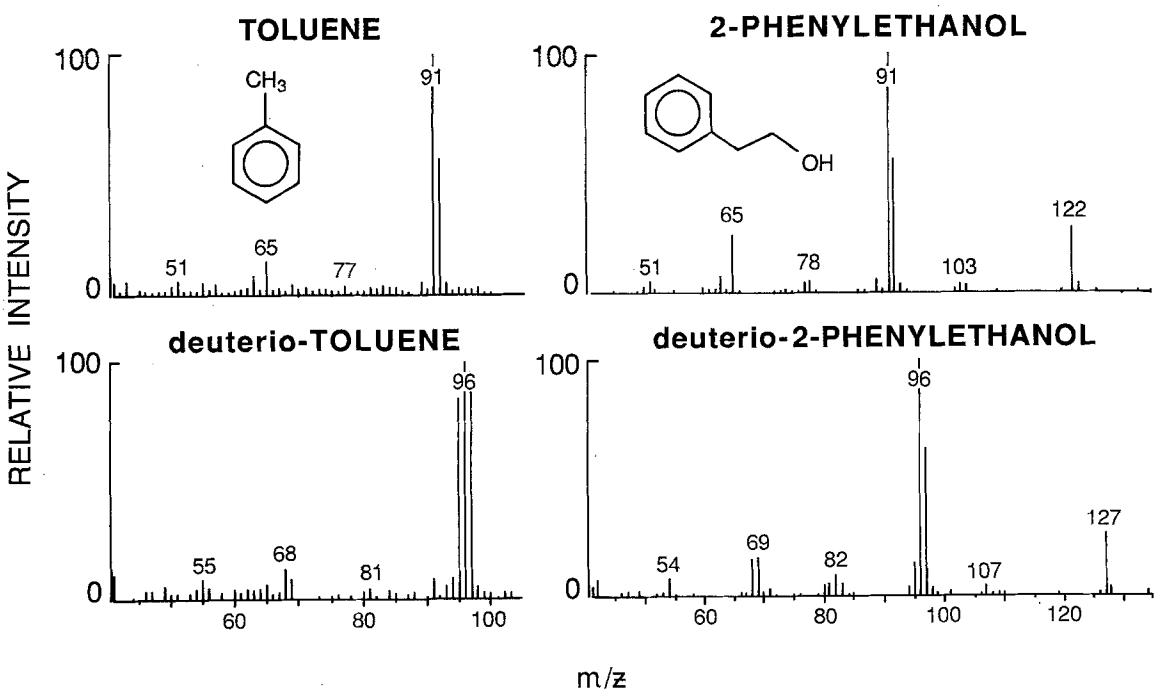
Results and discussion

Wild and axenic beetles of both sexes treated with deuterio-phenylalanine contained deuterio-toluene (fig., table); extracts of males also contained deuterio-2-phenylethanol (fig.). Neither deuterium-labelled compound was detected in untreated or in solvent-treated control beetles or in the phenylalanine solution used for topical treatment. The mean amounts of toluene present in different treatment groups did not differ significantly ($p > 0.05$; table), but the results suggest that axenically reared beetles of both sexes contained more of both unlabelled and deuterium-labelled toluene.

We conclude that both male and female *Ips pini* convert phenylalanine to toluene and that males also produce 2-phenylethanol. The latter compound has been previously identified in another scolytid beetle, *Ips paraconfusus*[22], and detected in volatiles from yeasts isolated from *I. typographus*[18] and other bark beetle species[15]. Our study on axenically-reared *I. pini* individuals, which lack culturable microorganisms, indicates that the beetles themselves are able to convert phenylalanine to toluene and 2-phenylethanol. Moreover, axenically-reared beetles tended to produce more toluene than wild beetles (table), suggesting that symbiotic microbes are probably not involved in phenylalanine conversion.

The small amounts of toluene in extracts (table) might be explained by the immediate release of this potentially harmful metabolite. The 5 µg of topically applied L-phenyl-d_5-alanine surpassed the internal content of naturally occurring phenylalanine in insects[19,20], but resulted in only small amounts of deuterio-toluene in the beetles, likely indicating a rather moderate penetration of deuterio-phenylalanine through the cuticle.

The adaptive significance of the production of toluene from phenylalanine is unclear. However, high levels of



Mass spectra of unlabelled and deuterium-labelled toluene and 2-phenylethanol detected in extracts of *Ips pini* treated with L-phenyl-d_5-alanine.

Amounts of unlabelled and deuterium-labelled toluene in extracts of *I. pini* treated topically with deuterium-labelled L-phenyl-d_5-alanine

Sex	Origin of beetles	No. of replicates 8–14 beetles/repl.	Amount of toluene per beetle [ng] ($\bar{X} \pm SE$)[1] unlabelled	labelled
Male	Wild	5	3.35 ± 1.91	0.15 ± 0.04
	Axenically reared	4	8.35 ± 4.72	0.41 ± 0.13
Female	Wild	2	0.83 ± 0.07	0.02 ± 0.01
	Axenically reared	3	7.98 ± 4.05	0.32 ± 0.11

[1] No significant difference between means within either column, Student Newman Keuls test, $p > 0.05$.

phenylalanine adversely affect the performance of aphids[25,26] and induce them to leave their feeding sites[27]. High levels of phenylalanine are likely also present in the phloem/(sapwood) diet of bark beetles as lignin synthesized from phenylalanine[28,29] comprises up to 20% of pine phloem[30]. The large amounts of toluene emitted from gallery systems of *I. pini* may result from specialized enzymatic systems responsible for processing excessive amounts of ingested phenylalanine.

It was hypothesized that toluene could be a pheromone in *I. pini*. However, a series of field experiments in which toluene was tested alone or in combination with the known pheromone, ipsdienol, failed to disclose consistent toluene-induced behaviour. Field testing of 2-phenylethanol as a candidate pheromone has not been conducted at this time, but it reportedly acts as a weakly-attractive pheromone for *I. paraconfusus*[22].

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5 Bernays, E. A., and Woodhead, S., J. Insect Physiol. *30* (1984) 489.
6 Brunet, P. C. J., Insect Biochem. *10* (1980) 467.
7 Munn, E. A., Feinstein, A., and Greville, G. D., Biochem. J. *124* (1971) 367.
8 Kramer, S. J., Mundall, E. C., and Law, J. H., Insect Biochem. *10* (1980) 279.
9 Hu, J.-S., and Chen, C.-M., Acta ent. sin. *30* (1987) 31.
10 Hatfield, L. D., Frazier, J. L., and Ferreira, J., Physiol. Ent. *7* (1982) 15.
11 Barber, J. T., Ellgard, E. G., and Plaster, B. C., J. med. Ent. *20* (1983) 641.
12 Meinwald, J., Koch, K. F., Rogers, J. E., and Eisner, T., J. Am. chem. Soc. *88* (1966) 1590.
13 Weatherston, J., and Percy, J. E., Insect Biochem. *6* (1976) 413.
14 Gries, G., Pierce, H. D. Jr, Lindgren, B. S., and Borden, J. H., J. econ. Ent. *81* (1988) 1715.
15 Brand, J. M., Schultz, J., Barras, S. J., Edson, L. J., Payne, T. L., and Hedden, R. L., J. chem. Ecol. *3* (1977) 657.
16 Conn, J. E., Borden, J. H., Hunt, D. W. A., Holman, J., Whitney, H. S., Spanier, O. J., Pierce, H. D. Jr, and Oehlschlager, A. C., J. chem. Ecol. *10* (1984) 281.
17 Hunt, D. W. A., PhD thesis. Simon Fraser Univ., Burnaby, B.C., Canada 1987.
18 Leufvén, A., Bergström, G., and Falsen, E., J. chem. Ecol. *10* (1984) 1349.
19 Barras, S. J., and Perry, T. J., USDA For. Serv. Gen. Tech. Rept. SO-10 (1975)
20 Leach, J. G., Orr, L. W., and Christiansen, C., J. Agric. Res. *49* (1934) 315.
21 Whitney, H. S., and Spanier, O. J., Can. Ent. *114* (1982) 1095.
22 Renwick, J. A. A., Pitman, G. B., and Vité, J. P., Naturwissenschaften *63* (1976) 198.
23 Rilling, G., Rapp, A., Steffan, H., and Reuther, V. K. H., Z. ang. Ent. *77* (1974) 195.
24 Jabbar, A., and Strang, R. H. C., Comp. Biochem. Physiol. B *78* (1984) 453.
25 v. Emden, H. F., in: Perspectives in Aphid Biology, p. 54. Ed. A. D. Lowe. Entomological Society of New Zealand, Bulletin No. 2, 1973.
26 Manoukas, A. G., Z. ang. Ent. *91* (1981) 309.
27 Havlickova, H., J. appl. Ent. *103* (1987) 142.
28 Freudenberg, K., and Neish, A. C., Constitution and Biosynthesis of Lignin, p. 129. Springer-Verlag, New York 1968.
29 Hess, D., Pflanzenphysiologie: Molekulare und biochemisch-physiologische Grundlagen von Stoffwechsel und Entwicklung, p. 379. Verlag Eugen Ulmer, Stuttgart 1976.
30 Côté, W. A. Jr, Simson, B. W., and Timell, T. E., Svensk Papperstidning årg. *69* (1966) 547.

0014-4754/90/030329-03$1.50 + 0.20/0