It is known that the pituitary is also effective in stimulating the lens-forming transformation of the outer cornea, likewise the neural retina, the regenerating limb, the limb bud and its blastema, the tentacle blastema and the spinal ganglia[7,16,17]. These data have been explained by assuming that, in addition to the retinal factor, a pituitary factor and a neurotrophic factor, produced by the ganglion cells and also by dedifferentiated cells of buds and blastema, are responsible for promoting the lens-forming transformations of the outer cornea[17,18]. Similar factors could be responsible for triggering the retinal transdifferentiation of the iris.

The available data concerning the lens-forming transformation of the outer cornea and the retinal transdifferentiation of the iris could be explained by assuming that the influence exerted by the triggering factors is permissive in nature, allowing the already-committed responding tissues (outer cornea, dorsal iris) to realize their latent intrinsic developmental capacities (see also Bosco[8]). Therefore, whereas the outer cornea is always transformed into lens, the dorsal iris, under the influence of the same tissues, always transdifferentiates into neural retina. Alternatively, one could suggest that different factors, instructive in nature, are responsible for promoting the lens-forming transformations of the outer cornea and the retinal transdifferentiation of the iris. In this hypothesis it is necessary to admit that factors promoting lens- and retinal-transdifferentiation are simultaneously present in all the different inducing tissues. However, this hypothesis seems to be less likely than the first.

As far as the identity of the inducer is concerned, the pituitary could exert its inducing effect by releasing hormones. However, the available in vitro data concerning the influence of bovine pituitary hormones on lens regeneration from newt iris epithelium failed to demonstrate a link between activity stimulating lens-regeneration and purified hormonal activity[19,20].

On the other hand, it could be argued that the pituitary factor is a neural growth factor. In this connection, it is interesting to note that the mitogenic protein called glial growth factor (GGF) originally purified from bovine pituitary[21] has recently also been detected in newt brain extracts[22]. It has been demonstrated that GGF is present in the innervated blastema of the newt regenerating limb, and is lost on denervation. A role of GGF in the nerve-dependent proliferation of the blastemal cells has been suggested[22].

1 Freeman, G., J. exp. Zool. *154* (1963) 39.
2 Bosco, L., Filoni, S., and Cannata, S., J. exp. Zool. *209* (1979) 261.
3 Filoni, S., Bosco, L., and Cioni, C., Acta embryol. morph. exp. *2* (1981) XXVI.
4 Filoni, S., Bosco, L., and Cioni, C., Acta embryol. morph. exp. *3* (1982) 15.
5 Filoni, S., Bosco, L., Cioni, C., and Venturini, G., Experientia *39* (1983) 315.
6 Reeve, J. G., and Wild, A., J. Embryol. exp. Morph. *48* (1978) 205.
7 Reeve, J. G., and Wild, A., J. Embryol. exp. Morph. *64* (1981) 121.
8 Bosco, L., Differentiation *39* (1988) 4.
9 Filoni, S., Bosco, L., and Cioni, C., Acta embryol. exp. *3* (1976) 319.
10 Bosco, L., Filoni, S., and Paglioni, S., J. exp. Zool. *216* (1981) 267.
11 Cioni, C., Filoni, S., Aquila, C., Bernardini, S., and Bosco, L., Differentiation *32* (1986) 215.
12 Cioni, C., Filoni, S., Bosco, L., Aquila, C., and Bernardini, S., Experientia *43* (1987) 443.
13 Nieuwkoop, P. D., and Faber, J., Normal Table of *Xenopus laevis*. Daudin-North-Holland Publ., Amsterdam 1956.
14 Sacerdote, M., Z. Anat. Entwickl. Gesch. *134* (1971) 49.
15 Clayton, R. M., Adv. exp. Biol. Med. *158* (1982) 23.
16 Waggoner, P. R., J. exp. Zool. *186* (1973) 97.
17 Bosco, L., Filoni, S., and Cioni, C., J. exp. Zool. *233* (1985) 221.
18 Filoni, S., Bosco, L., Cioni, C., and Burani, P., J. exp. Zool. *230* (1984) 409.
19 Connelly, T. G., Differentiation *16* (1980) 85.
20 Cuny, R., and Zalik, S., Exp. Eye Res. *41* (1985) 629.
21 Lemke, G. E., and Brockes, J. P., J. Neurosci. *4* (1984) 75.
22 Brockes, J. P., and Kintner, C. R., Cell *45* (1986) 301.

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Hydrocarbons in tarsal glands of *Bombus terrestris*

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Summary. Chemical components of tarsal glands of *Bombus terrestris* workers were identified by combined gas chromatographic/mass-spectrometric analysis. The 17 components are exclusively saturated and unsaturated hydrocarbons.
Key words. Bombus; tarsal gland; forage-marking; hydrocarbons.

In honeybees, tarsal glands are situated in the pretarsae of all legs and were first described by Arnhart[1] (Arnhart's glands). Although honeybees are one of the best examined insects, a comprehensive chemical analysis of tarsal glands is still lacking. Chauvin[2] examined infrared spectra of the gland compounds and found a waxy composition with aromatic substances. In bumblebees, tarsal glands are located in the pretarsae too and are less devel-

oped [3]. During recent work with foraging bumblebees, in which scent-marked artificial food sources were used [4], we found that previously-visited food sources (possibly marked) were more efficiently located. In a first step tarsal glands of *Bombus terrestris* were examined; the results are presented here.

Bumblebee workers (*B. terrestris*) were taken from different colonies and kept in flight cages in a climatically controlled greenhouse. Samples taken from tarsal glands of workers kept in cages together with males showed contamination with labial-gland marking-components of the males. To get the tarsae of the bees as clean as possible, workers of male-free colonies were kept in clean cages for one day before preparation of the glands. The claw of the tarsal which touches the substrate was cut off and discarded (fig. 1) and the remainder of the tarsal was cut off and placed in pentane. Thirty tarsal segments (= 1.5 mm in length) were removed from 5 animals and placed in 0.2 ml of pentane per sample. To make sure that gland components were completely extracted, samples were frozen in fluid N_2 and ground in a mortar. For combined gas chromatographic/mass-spectrometric analysis a VARIAN 3400 gas chromatograph connected to an ION TRAP DETECTOR (Finnigan MAT) was used.

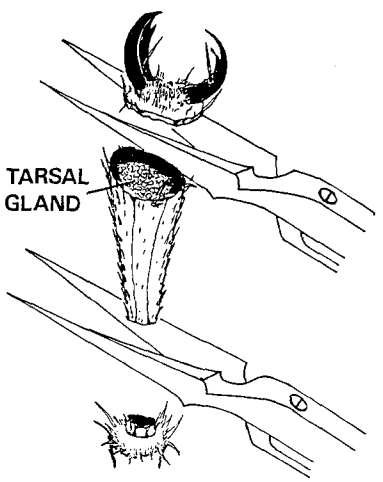

Figure 1. Preparation of tarsal gland by cutting off the pretarsus.

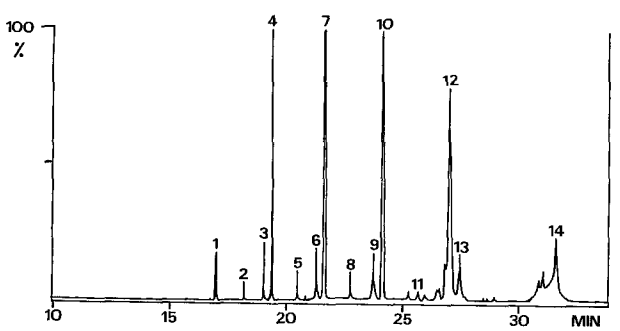

Figure 2. Chromatogram of tarsal gland extracts; peak-numbers – refer to the table.

Tarsal gland components of *B. terrestris*

Tarsal gland components	MW	ng/gland
1 Heneicosene *	294	< 1
2 Heneicosane	296	4
3 Docosane	310	1
4 Tricosene *	322	4
5 Tricosane	324	22
6 Tetracosane	338	2
7 Pentacosene *	350	2
8 Pentacosane	352	53
9 Hexacosane	366	2
10 Heptacosene *	378	2
11 Heptacosane	380	30
12 Octacosane	394	1
13 Nonacosene *	406	10
14 Nonacosane	408	3
15 Triacontane	422	< 1
16 Hentriacontene *	434	3
17 Hentriacontane	436	2

* = mixtures
double-bond positions
Heneicosene – 9, 10
Tricosene – 7, 8, 9, 10, 11
Pentacosene – 6, 7, 8, 9, 10, 11, 12
Heptacosene – 5, 6, 7, 8, 9, 10, 11, 12, 13
Nonacosene – 7, 8, 9, 10, 11, 12, 13, 14
Hentriacontene – 9, 10, 11, 12, 13, 14, 15
(main positions underlined)

Temperature-programming: 120 °C for 1 min – heating 8 °C/min – 280 °C for 15 min; Column: 30 m × 0.25 mm ID J&W Scientific DB-1; Carrier-gas: helium.

15 samples with tarsal gland extracts were analyzed. To estimate the amount of the components per sample (= 30 glands) an internal standard (nonadecane 10^{-4} mg/ml) was added to three samples. The chromatograms do not vary qualitatively in the range of the components identified. There are only slight differences in the relative proportions of certain compounds. Figure 2 shows a typical chromatogram.

The components of the tarsal glands so far identified are exclusively alkans and alkens, as was shown by their mass-spectras. Some of the alkens are mixtures of positional and/or geometrical isomers. The double-bond positions were carried out with the help of DMDS – derivatization [5] and mass-spectrometric data. In the table the components are listed with molecular weight and information about the amount per gland.

Tarsal glands as sources of pheromones have been investigated in ants [6] and honeybees [7, 8]. In bumblebees, as in honeybees, the tarsal glands produce relatively large amounts of wax [2]. In the analyzed samples of tarsal glands of *Bombus terrestris*, no aromatic substances were found except in those glands contaminated with male-marking pheromones. To estimate the role of hydrocarbons as chemical signals there must first be a chemical analysis of the deposited scent. Artificial food sources which are marked by bumblebee workers [4] will be used for the collection of the deposit. In addition, chemical analysis of different glands of bumblebees as sources of forage-marking signals is in progress.

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1 Arnhart, L., Arch. Bienenkde *5* (1923) 37.
2 Chauvin, A., Insect. soc. *9* (1962) 1.
3 Cruz Landim, C., and Staurengo, M. A., Proc. V. int. Congr. I.U.S.S.I. (1965) 219.
4 Schmitt, U., and Bertsch, A., Oecologia *82* (1990) 137.
5 Francis, G. W., and Veland, K., J. Chromat. *219* (1981) 379.
6 Hölldobler, B., and Palmer, J. M., Naturwissenschaften *76* (1989) 385.
7 Butler, C. G., Fletcher, D. J. C., and Watler, D., Anim. Behav. *17* (1969) 142.
8 Ferguson, A. W., and Free, J. B., J. apic. Res. *18* (1979) 128.

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Synergism of a natural insect growth inhibitor is mediated by bioactivation

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Summary. Toxic plant allelochemicals are widespread in nature, but their mechanisms of action are largely unexplored. We report an example of bioactivation of a natural product, β-asarone, that is mediated via insect mixed-function oxidases (MFO's), enzymes usually involved in detoxication processes. Bioactivity of β-asarone is synergised by menthol, a MFO inducer, and antagonized by the MFO inhibitor piperonyl butoxide. Formation of a bioactive epoxide was confirmed by the identification of asarone epoxide and asarone diol in the insect excreta. These experiments represent the first demonstration of synergism between two natural products (β-asarone and menthol) where the mechanism involves induction of enzymes usually involved in detoxication.
Key words. β-Asarone; bioactivation; MFO's; *Peridroma saucia; Acorus calamus.*

The bioactive constituent of sweetflag, *Acorus calamus* L. (Araceae) which induces antigonadal, antifeedant, and growth inhibitory effects[1-3] in insects, is 2,4,5-trimethoxy propenylbenzene (β-asarone, fig. 1, I). It had previously been established that both the double bond in the alkyl side chain and its *cis* configuration are structural requisites for bioactivity[1,4]. However, as the mode of action for the various deleterious effects of β-asarone is not known, we investigated the mechanism underlying the growth inhibitory activity of β-asarone following topical application to variegated cutworm (*Peridroma saucia*) (Lepidoptera: Noctuidae) larvae.

Early fourth instar larvae (11.5 ± 0.3 mg b.wt) were topically treated with β-asarone (isolated from sweetflag, *Acorus calamus*[3]) at doses of 5–30 µg/larva in 1 µl of acetone (20 larvae/treatment, controls treated with carrier alone). Treated and control larvae were fed artificial diet (Bioserv. Inc., Frenchtown, N. J., USA) for 72 h, after which insects, remaining food and frass were dried at 60 °C to constant weight and weighed. Relative growth rate (RGR) and efficiency of conversion of ingested food (ECI) were subsequently calculated[3]. This experiment showed that reduction in gross dietary utilization (ECI) of treated larvae was significantly dose dependent ($p < 0.05$, linear regression). A dose-dependent reduction in RGR concomitant with decreasing ECI (fig. 2) indicated that inhibition of growth was primarily attributable to increased metabolic cost.

β-Asarone shows a structural resemblance to the precocenes[5], plant natural products with anti-juvenile hor-

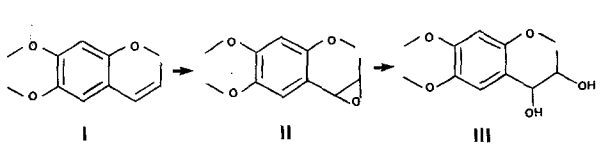

Figure 1. Proposed pathway of metabolic bioactivation of β-asarone in *P. saucia* larvae. I = β-asarone; II = asarone epoxide; III = asarone diol.

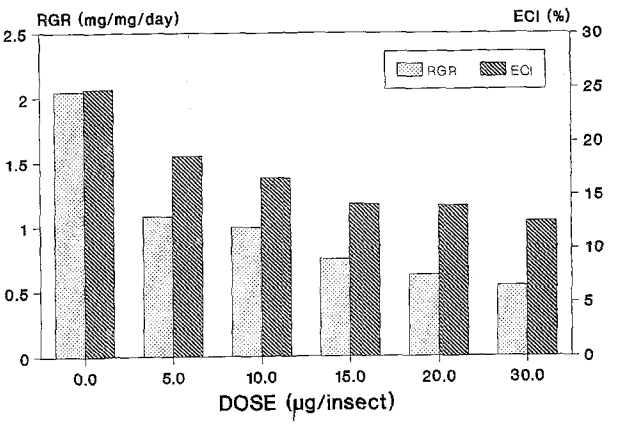

Figure 2. Effect of topically-applied β-asarone on the relative growth rate (RGR) and gross dietary ultilization (ECI) of *P. saucia* larvae fed on artificial diet.