

Fig. 1. Release of group recruitment in *L. chinensis* by laying an artificial trail with a mixture of venom and dorsal gland secretion.

the dorsal gland was inhibited with wax, these animals were not able to recruit more than one or two nestmates, although they used their intact sting for laying trails. These experiments indicated that our hypothesis on the function of the dorsal gland was correct.

Moreover, the pattern of group recruitment is an eminent clue to understanding the nest moving of *L. chinensis*. The emigration of the entire colony can be initiated by a single scout who has found a new nest site. We are able to induce large groups of ants to leave the nest by laying a trail with a mixture of the secretion of the venom and dorsal glands through the nest entrance and at the same time offering large quantities of it in the nest. In a few experiments even single pupae were transported, which is characteristic for the beginning of nest moving. This did not happen when only one of the gland secretions was offered. We observed that in the course of each normal colony emigration, without exception, one or more animals chirped with their gastral stridulation organ. Scouts with blocked dorsal glands

who had found a new nest site were not able to form an emigration group in the old nest, although they stridulated and laid trails with their stings. When, on the other hand, the stridulation organs were blocked, but the dorsal glands left intact, the scouts did not lose their group recruiting ability in nests ready for emigration. Their ability to initiate colony emigration, however, was strongly weakened. In only a few experiments was a regular colony emigration initiated by these workers. In these cases we observed other emigrating workers chirping.

Thus, the secretion of the dorsal gland is a crucial signal for recruiting groups for group predation, as well as for colony emigration. For a regular colony emigration, the additional mechanical signal of chirping is necessary.

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## 2-Ethyl-1,6-dioxaspiro[4.4]nonane, Principal Aggregation Pheromone of *Pityogenes chalcographus* (L.)

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Populations of the "Kupferstecher", *Pityogenes chalcographus* (L.), infest Norway spruce, *Picea abies* (L.), recently creating a serious pest problem in European spruce stands. These tiny beetles aggregate like

other species of *Pityogenes* [1] on standing trees or slash of freshly cut trees, in response to a pheromone released by the host-selecting male beetles [2].

The isolation of the pheromone followed

the principle of differential diagnosis [3]. Only male beetles feeding in the phloem tissue of host material or treated with juvenile hormone analogues (JHA) [4] were highly attractive to male and female beetles in flight [5]. Gas chromatographic analyses of the volatile content of such beetles revealed consistently three major peaks which were not detectable in unfed or untreated males or in female beetles treated in the same manner.

For chemical identification, approximately 100 000 beetles of both sexes were collected from cages as they emerged from naturally infested host material. Subsequently the beetles were treated with JHA ZR 233 (ethyl-3,7,11-trimethyl-2-dodecenoate), employing a novel technique [6]. This treatment was highly efficient in providing a relatively pure source of the candidate compounds.

The three male-specific peaks (Carlo-Erba 2100; 50 m, 0.25 mm i.d., stainless steel column with Marlophen 87, programmed from 50–140 °C at a rate of 3 °C/min) were subjected to mass-spectral analysis. The first substance was identified as 1-hexanol by comparison with an authentic sample on a Varian MAT 111 GC-MS-coupling system.

The two others, which were eluted as a double peak 3 min after 1-hexanol showed parent ions at *m/e* 156 (2%) and a similar fragmentation pattern on GC-MS, obviously representing two isomeric compounds: 127 (100;100), 87 (64;82), 85 (60;76), 43 (46;79), 56 (42;69), 55=57 (38;60), 41 (31;51), 97 (30;35), 42 (28;42), 69 (29;30), 98 (26;35), 81 (20;22), 39 (16;19), 73 (11;14).

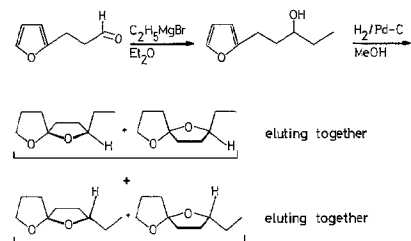
From pentane extracts, the substances were isolated as a 1:1 mixture by preparative GLC (Perkin-Elmer F 21; 9 m, 8 mm i.d., stainless steel column with 5% XE 60 on Chromosorb G, 45–60 mesh). The molecular formula  $C_9H_{16}O_2$  was obtained by high-resolution mass spectrometry, and the base peak was determined to be  $C_7H_{11}O_2$ , indicating the loss of an ethyl group from the molecule (Varian MAT SM 1). The  $^1H$ -NMR spectrum showed three characteristic signals at  $\delta=4.07$ , 3.89, and 3.70 (Bruker WH 270, 30 000 scans), each of which represented one proton in an  $\alpha$ -position to oxygen, thus suggesting a bicyclic ketal structure analogous to known bark-beetle pheromones [7–9]. Though the fragmentation patterns of the unknown substances resembled those of the 6,8-dioxabicyclo[3.2.1]octanes and al-

Table 1. Field response of *P. chalcographus* to tree trunk-simulating olfactometers baited with chalcogran. Mooswald near Freiburg i.Br. (Germany), 22. April 1977

Test material	Repetitions	Average number (and range) of beetles responding	Sex ratio (♂/♀)
Chalcogran + spruce bark	4	23 (7-31)	5.4:1
30 ♂ feeding in spruce log	4	29 (16-50)	4.5:1
Spruce bark	4	1 (0-2)	—
Control (empty)	4	0	—

kyl-2,9-dioxabicyclo[3.3.1]nonane [10], the intensities of some fragment ions ( $M^+ - \text{alkyl} = 100\%$  etc.) differed considerably from all known members of these classes. Also 3-ethyl-6,8-dioxabicyclo[3.2.1]octane, which was prepared for comparison, did not match the above values. From the base peak at  $m/e$  127 it was concluded that the ethyl group should be adjacent to oxygen in a rather stable system, and indeed the *E,Z*-isomers (reference plane *Q* is the alkylated furan ring [11]) of 2-ethyl-1,6-dioxaspiro[4.4]nonane, belonging to a bicyclic ketal system which was previously found in *Chrysanthemum* spp. [12] and in hop oil [13] were found to be identical in all respects with the natural compounds.

A racemic mixture of the two pairs of diastereomers was prepared by Grignard reaction of 3-(2-furyl)-propionaldehyde [14], followed by reductive cyclization [15] under 1 psi  $H_2$  with 1% of 10%-Pd/C catalyst and purified by preparative GLC.



The stereo-chemistry of the naturally occurring compounds is not yet clarified, but work is in progress to prepare the four possible isomers for bioassay.

The mixture of isomers proved to be biologically active in the field. Bioassays using sleeve olfactometers [16] in a spruce forest near Freiburg indicated that the synthetic mixture (in combination with fresh spruce bark) was almost as attractive as a spruce log in which 30 males beetles had been feeding for 48 h (Table 1). Since relatively large quantities of the synthetic material (~15 mg/h) were used in the bioassay, a second component may be necessary for maximum response. When 1-hexanol was added to the synthetic mixture in field

bioassays, no apparent effect was observed. However, it is also possible that the enantiomeric composition of the isomers is critical for maximum beetle response.

As the compound was first isolated from *P. chalcographus*, we suggest the trivial name "chalcogran" for 2-ethyl-1,6-dioxaspiro[4.4]nonane.

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## Water Balance and Renal Adaptations in Four Palearctic Hamsters

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It was demonstrated that four species of palearctic hamsters have different capacities for water conservation when their water supply is severely limited. A correlation was established between water economy and geographical range of these hamsters.

Adult European hamsters (*Cricetus cricetus*,  $n=24$ ), Golden hamsters (*Mesocricetus auratus*,  $n=35$ ), Chinese hamsters (*Cricetulus griseus*,  $n=52$ ), and Djungarian hamsters (*Phodopus sungorus*,  $n=48$ ) were housed and bred at room temperature and in natural daylight from July 1975 to June 1977. For a urine-concentrating test, adult hamsters of each species ( $n=10$ ) were provided with a mixed diet of sunflower seed, apples, and lettuce ad lib. until their weight became stable ( $T_a$  22 °C, LD 12:12 h). No drinking water was available. During a period of four months, urine concentrations of 200 samples from each group were determined cryoscopically. The average urine concentration (all data are means

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and standard deviations) was lowest in the European hamster ( $735 \pm 99$  mOsmol/l), followed by the Golden hamster ( $1266 \pm 232$ ), the Chinese hamster ( $1517 \pm 236$ ), and the Djungarian hamster ( $1519 \pm 204$ ). To measure the kidney's maximum ability to concentrate, the hamsters were put on a dry diet of soybeans over a period of 8 days ( $T_a$  22 °C,  $55 \pm 10\%$  r.h.). The values increased to a mean of  $2986 \pm 356$  in *Cricetus*,  $2861 \pm 212$  in *Mesocricetus*,  $3794 \pm 480$  in *Phodopus*, and  $3107 \pm 372$  in *Cricetulus*. (Maximum values were 3840, 3440, 4572, 4592 mOsmol/l.) Although these values are very high, none of the hamsters reached the urine concentrations of typical desert rodents [1].

The response of body weight to water deprivation with a diet of sunflower seed is summarized in Figure 1a. The little decrease in weight in the European hamsters agrees with earlier observations [2]. Surprisingly, the Chinese hamsters lost weight