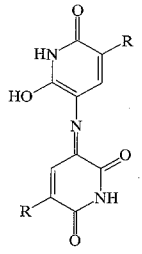
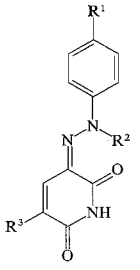


1: R = β-D-ribopyranosyl
2: R = CH_3



3: $R^1 = R^2 = H$, R^3 = β-D-ribopyranosyl
4: $R^1 = NO_2$, $R^2 = H$, R^3 = β-D-ribopyranosyl
5: $R^1 = R^2 = H$, $R^3 = CH_3$
6: $R^1 = NO_2$, $R^2 = H$, $R^3 = CH_3$
7: $R^1 = H$, $R^2 = R^3 = CH_3$

$C_{16}H_{17}N_3O_6$, m.p. 265 °C (resp. *4*, $C_{16}H_{16}N_4O_8$, m.p. 248 °C, decomp.), which has quite the same spectral data (UV, IR, MS, NMR) as 3-methyl-5-phenylhydrazono-2,6-pyridindione (*5*) [5] (resp. *6*) derived from *2*, and 5-(N-methyl-N-phenylhydrazono)-3-methyl-2,6-pyridindione (*7*).

3, *4*, *5*, and *6* are Z-isomeres and possess strong hydrogen bonds between 6-CO and NH of the phenylhydrazone. On ozonolysis of *3* D-ribose is obtained; oxidation with periodate, NMR and ORD spectra prove a β-D-ribopyranose residue. Boiling in 1 *N* hydrochloric acid effects isomerization of the β-pyranose residue in *3*; the four isomeric 5-phenylhydrazono-3-D-ribosyl-2,6-pyridindiones formed on this treatment can easily be separated on silica gel impregnated with boric acid. *3* and its anomer show Cotton effects at 415 nm (DMSO) of opposite sign (*3*: negativ) in their ORD curves.

As ozonolysis of the minor pigments again yields D-ribose only, "Amylocyanin" obviously is a mixture of ribosyl-isomere diazaphenol-indophenols. The results of isolation and determination of configuration of all the components will be published elsewhere.

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Field Response to a New Pheromonal Compound Isolated from *Ips typographus*

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The bark beetle *Ips typographus* (Linnaeus), one of the most aggressive and serious pests of spruce in Eurasia, has previously been shown to respond to a mixture of stereo-isomeric verbenols and 2-methyl-6-methylene-2,7-octadiene-4-ol (ipsdienol) [1]. These compounds and 2-methyl-6-methylene-7-octene-4-ol (ipsenol) [2] are present in several *Ips* species from the Nearctic as well as the Palearctic region [3], and they are the only biologically active insect-produced compounds known from *Ips*. From the hindgut of male *Ips typographus* initiating their galleries in standing, healthy trees we have detected an additional compound, 2-methyl-3-butene-2-ol (methylbutenol), which admixed with *cis*-verbenol and ipsdienol strongly increases the response of flying male and female beetles in the field.

An ether extraction of the hindguts was analyzed using the technique of combined gas chromatography–mass spectrometry (GC-MS). The gas chromatographic glass column, 2.44 m × 2.54 mm, was packed with 10% FFAP on Varaport 30 and kept at 70–230 °C; MS source 250 °C and 70 eV. The retention times and the mass spectra of the active compounds were in complete agreement with those of authentic material [4]. In the case of the volatile compound 2-methyl-3-butene-2-ol the best results were obtained by transferring the hindguts directly to a 0.5-ml glass tube which was subsequently sealed with a rubber septum. The tube was heated at 100 °C for 1 min, the volatiles removed with a hypodermic syringe and injected into the GC-MS system.

Field tests were performed in forests of Norway spruce at Eidskog, County of Hedmark, in Southern Norway during May and June 1976. We used 10 olfactometers, always five units together, during the test period. Synthetic test materials of insect-produced compounds and host volatiles were combined in various proportions in 25 different tests, which were repeated at least 5 times. Test periods varied between 30–90 min depending on time of the day and flight activity. A total of 32000 beetles were trapped. The isomeric verbenols and ipsdienol were commercial materials supplied by A/S Borregaard, Sarpsborg, Norway. The release of pheromones was approximately as follows: methylbutenol 0.4 mg/h, ipsdienol and verbenols 0.02 mg/h at 20–25 °C.

Each of the compounds alone attracted only a few beetles. In every test the combination of *cis*-verbenol, methylbutenol, and ipsdienol attracted the most beetles (Table 1); no enhanced response was recorded by addition of an extract of volatiles from newly cut spruce stems, or when α-pinene, β-pinene, or myrcene were admixed.

The attractiveness of infested spruce bolts was compared to that of the test materials. The bolts (15 × 40 cm) were infested with 40 males two days before use. The controls were traps charged with newly cut bolts and traps without bait of any kind. No significant difference was observed between males and females in their response to the various combinations of pheromones (Table 2).

Both methylbutenol and *cis*-verbenol were present in male beetles initiating boring

Table 1. Number of beetles trapped in response to synthetic pheromones (3–4 June)

Material tested	Mean response of 5 runs (1 h)	Range	Sex ratio ♂:♀
Methylbutenol + *cis*-verbenol + ipsdienol	103.2	187-39	1:1.8
Methylbutenol + *cis*-verbenol	52.6	137-15	1:2.1
Methylbutenol + ipsdienol	21.8	41-10	1:2.4
Methylbutenol	5.6	10-4	1:2.5
Control	3.4	10-0	1:2.1

Table 2. Number of beetles trapped in response to synthetic pheromones, to male beetles boring in fresh logs, and to fresh logs only (10 June)

Material tested	Mean response of 5 runs (0.5 h)	Range	Sex ratio ♂:♀
Methylbutenol + *cis*-verbenol + ipsdienol	77.8	169-22	1:2.1
Methylbutenol + *cis*-verbenol	56.2	127-7	1:1.7
Infested bolt 40 ♂♂ in 2 days	6.6	10-4	1:1.2
Fresh bolt	3.8	8-2	1:2.1
Control	1.8	5-0	1:0.9

in standing trees and fighting the resin flow; hence, it suggests that they are the primary aggregation pheromones of *Ips typographus*, which attract the large population necessary to overcome the resistance of standing, healthy trees.

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Electrophysiological Recordings from the Lateral Ocelli of *Drosophila*

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Quite a number of mutations affecting receptive or neural structures of the compound eye of *Drosophila melanogaster* have been isolated and the effect of these mutations on anatomy and physiology of the compound eye and on behavior has been examined [1–3]. However, the effect of these mutations on the fly's frontal eyes, the ocelli, has not yet been investigated. This paper describes a method for electrophysiological recording from the ocelli of *Drosophila* and presents some preliminary findings.

Drosophila melanogaster (white-eyed, if not specified otherwise) were immobilized by chilling on ice and fixed in an upright position by waxing the tips of the laterally extended legs to a cover slip. The head was waxed to the cover slip with its caudal surface in an approximately horizontal position. Additional mechanical stabilization of the head and thorax was achieved by means of lateral wax bridges. An oscillating razor blade guided by a micromanipulator was used to remove a small piece of cuticle (ca. 100 µm diameter) from the caudal head surface. The dorsal edge of the cut just touched the lateral ocelli. Hoyle's saline [4] was applied to the head immediately while a wax barrier kept the abdomen and thorax free of saline. The tips of the micropipettes (filled with 2*M* KCl, 100–200 MΩ) were aimed just behind the ocellar lens, the site of the retinal cell layer [5, 6]. The electrode was advanced vertically in 2–5-µm steps. The penetration of cells was aided by inducing electronic oscillations at the tip of the electrode through overcompensation of the input capacitance of the electrometer.

The responses to rectangular flashes of white light (tungsten lamp) most frequently observed in ca. 40 animals are presented in Figure 1 and Table 1. Sometimes transitions from one response type to another during recording were found.

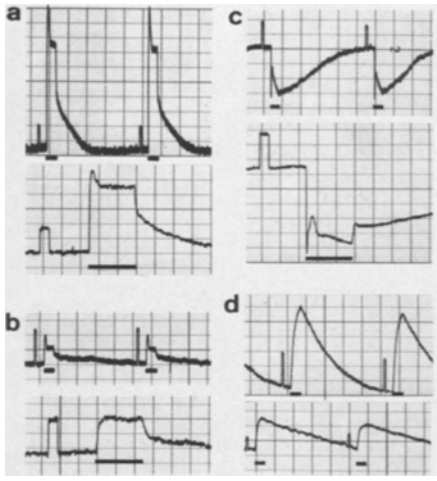

Fig. 1. Responses obtained in the lateral ocelli of *Drosophila*. (a) Type-A response (receptor potential), (b) type-B response, (c) type-C response, (d) type-D response. Bars indicate light stimulus. Calibration pulse: 0.1 s, 5 mV positive-going

The shape, polarity, amplitude, and preceding drop of baseline of the type-A response suggest that it may be an intracellularly recorded receptor potential of the ocellar photoreceptor. This is supported by the fact that the input resistance measured by applying 0.25-nA current pulses and observing the imbalance of the bridge

Table 1. Electrical responses to light flashes observed in the ocelli of *Drosophila*

Response type	Shape	Max. amplitude observed [mV]	Preceding shift of baseline[a]
A	Fast; positive-going; on-transient and plateau; at low intensities rectangular	30	negative
B	Fast; positive-going; rectangular, often on-transient	10	?
C	Negative-going; often spike-like on-transient	15 usually smaller	usually positive
D	Slow; positive-going; sometimes negative spike-like on-transient	20	usually negative

[a] DC shift of baseline on advancing the electrode before the described response could be observed

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