SPECIALIA

2-Phenylethanol, a Presumed Sexual Stimulant Produced by the Male Cabbage Looper Moth, *Trichoplusia ni*[1]

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Summary. A sex pheromone produced by male cabbage looper moths, *Trichoplusia ni* (Hübner), has been isolated from the genital scent brushes and identified as 2-phenylethanol. It is shown conclusively to elicit specific behavioural responses in the female (such as wing vibration and abdominal elevation), as determined by a novel behavioural laboratory bioassay. This is taken as further evidence that the male pheromone of *T. ni* acts as a sexual stimulant (aphrodisiac) prior to mating. 2-Phenylethanol represents the first identification of a genital scent brush pheromone in the family Noctuidae, and of a male pheromone in the subfamily Plusiinae.

The cabbage looper, *Trichoplusia ni* (Hübner), is a serious pest of cruciferous crops. In common with numerous species of the family Noctuidae, the adult male possesses glands attached to the 8th abdominal segment which are associated with tufts of hair[4,5]. These genital hairpencils, which are extruded and fanned out by the male as 'scent brushes' during courtship, have long been suspected to disseminate volatile pheromones sexually stimulating to the female[6-8].

SHOREY[9] was unable to demonstrate pheromonal stimulation of the female *T. ni* by the male in the laboratory, although GOTHILF and SHOREY[10] recently reported that exposure of the male's hairpencils next to the female during courtship causes her to vibrate her wings. GRANT[11,12] demonstrated electrophysiologically (by electroantennograms) that a substance on the male hairpencils was detected by the antennae of both male and female *T. ni*. However, although electroantennograms can be used to show that male (or female) pheromones are perceived peripherally by the antennal receptor cells of an insect, there is no alternative to using a behavioral assay to establish sexual stimulation[13]. This report provides additional evidence that the hairpencils of the male cabbage looper moth produce a volatile secretion which elicits overt sexual behavior in the female, and establishes its identity.

Laboratory-reared cabbage loopers[14] were sexed in the pupal stage, and the male and female pupae were kept in separate collapsible metal cages (26.9 × 26.3 × 26.9 cm) placed in adjacent rooms. 2 or 3 days following emergence, adult males were removed from the cage, the tip of the abdomen was squeezed gently to expose the scent brushes, and the brushes with the 8th abdominal segment were clipped into spectral grade pentane and ground in a mortar. Filtered extract from 250 males was freed of solvent at 15 mm pressure to give 1.5 g of pale yellow oil that was dissolved in hexane and stored at −10 °C until bioassayed. All electrophysiological and behavioural bioassay tests were conducted between midnight and 03.00 h (optimum for mating) in a dark room with a single light source in the form of a Kodak lamp with a red Safelight filter (Wratten series 1)[15]. The electroantennogram tests were carried out as described previously by ADLER[16,17] and GRANT et al.[18]. Nerve impulses from single pheromone receptors of the female *T. ni* were recorded either by tungsten electrodes (tip diameter <0.5 µm) inserted at the sensillum base, or by KAISSLING's method[19] of recording from the opened sensillum tip via a capillary electrode. All bioassays were performed with insects at least 48 h old.

At 16.30 h the Kodak lamp was turned on in the bioassay room and propped up against the cage containing virgin female moths; it remained in this position throughout the test period. Little flight activity was noted in the cage between midnight and 03.00 h; the insects tended to cling by their legs to the cage sides or roof, thus exposing the tarsi outside the cage. The test compound was presented to the female by means of a brush fashioned by gluing a short piece of multiple-strand string to one end of a Mulco sanitary hardwood applicator handle; after

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[4] M. JACOBSON, *Insect sex pheromones* (Academic Press, New York 1972), p. 93.
[5] M. C. BIRCH, in *Pheromones* (Ed. M. C. BIRCH; North-Holland Publ., Amsterdam 1974), p. 115.
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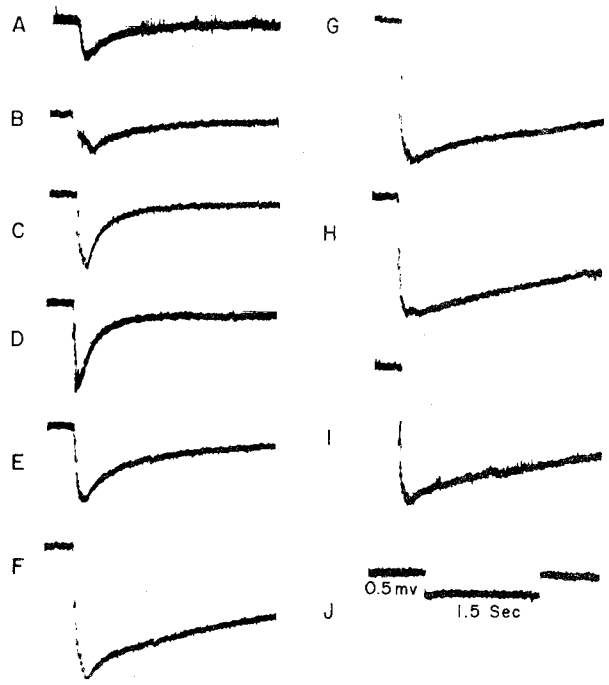
[19] K. E. KAISSLING, in *Biochemistry of Sensory Functions* (Ed. L. JAENICKE; Springer Verlag, Berlin 1974), p. 243.

the glue hardened the string was trimmed to a length of 12 mm. A 10-µl sample of a hexane solution of the test material was placed on the applicator brush tip, and this was then used to gently stroke the tarsi of the female looper. Behavioural stimulation by an active compound was evidenced within 2–7 sec by wing flutter and elevation of the tip of the abdomen. Cessation of tarsal stroking triggered an end to wing flutter, and the abdomen gradually assumed its normal position. In no case did a female show wing flutter and abdominal elevation when stroking was done with a tip treated only with hexane alone or untreated or with other (inactive) test compounds.

Electrophysiological and behavioural responses of female *T. ni* to 2-phenylethanol.

Dosage[a] (µg)	EAG amplitude[b] (mV)	Receptor responses (Imp/sec)[c]	Behavioural response[d]
0 (Control)[e]	0.9	3.4(15)	0
0.0001	0.75	–	0
0.001	1.0	2.5(6)	0
0.01	0.9	6.1(18)	0
0.1	1.4	13.9(20)	+
1.0	2.0	36.3(17)	++
10.0	1.9	59.5(8)	+++
100.0	2.0	71.0(2)	+++

[a] The designated amount of 2-phenylethanol was presented as a solution in 10 µl of pure hexane and the solvent was allowed to evaporate. [b] 5 recordings were made at each dose. [c] Mean number of nerve impulses within 1 sec of stimulation for 4 receptor cells of female Sensilla trichodea. Brackets indicate number of recordings. [d] 4 virgin females were tested for sexual response (replicated 5 times). 0 indicates none responded, + indicates 2 responded, ++ indicates 3 responded, and +++ indicates all responded. [e] Blank cartridge, or applicator brush treated only with hexane, respectively.



Electroantennograms (EAGs) from female *T. ni* antennae exposed to crude male hairpencil extract and to serial dilutions (in 10 µl of hexane) of the pure pheromone (2-phenylethanol). A) control (blank cartridge); B) 0.2 male equivalent of hairpencil extract; C–I) 2-phenylethanol at 0.0001, 0.001. 0.01, 0.1, 1, 10 and 100 µg, respectively; J) calibration.

The crude male extract (1.5 g) was chromatographed on a column (3 × 33 cm) of silicic acid (Bio-Sil HA; Bio-Rad Labs., Richmond, Calif.) and eluted successively with hexane-ethyl ether (90:10, 75:25 and 1:1), and ethyl ether. Strong electroantennograms (EAGs) were obtained with 10 µg of the material eluted only with hexane-ethyl ether (1:1); the EAG responses were 1.1–1.5 and 0.6–1.0 mV from females and males, respectively. The active eluate contained 4 mg of colorless liquid with a floral odor; gas chromatography on a nonpolar column (5% SE-30 on base-washed Chromosorb G; 0.32 cm × 2.4 m; 125 °C; gas flow 15 ml/min) and a polar column (5% Carbowax 20M TPA on base-washed Chromosorb G; 0.32 cm × 1.2 m; 128 °C; gas flow 20 ml/min) showed it to be a pure compound with retention times of 7.5 and 9.0 min, respectively. A strong behavioural response was obtained from female loopers with one male equivalent (16 µg) of this compound.

A mass spectrum of the pheromone obtained with a LKB-9000 gas chromatograph-mass spectrometer utilizing 0.75% SE-30 as the stationary phase at 85 °C had a molecular ion at *m/e* 122 and showed the following peaks (mass and percentage height given): 91 (100), 92 (63), 122 (33), 65 (14), 39 (6), 51 (5), 93 (5), 77 (5), 104 (2.5). This is the typical disintegration pattern of 2-phenylethanol, the base peak (*m/e*) of 91 corresponding to $C_6H_5CH_2^+$ (loss of CH_2OH)[20]. The isomeric alcohol, 1-phenylethanol, exhibits a totally different spectrum with a base peak at *m/e* 107. IR- and NMR-spectra were in complete agreement with the assignment of 2-phenylethanol, all spectra being identical with those of an authentic commercial sample. Authentic 2-phenylethanol elicited electroantennograms, single cell responses, and sexual excitation (Table) in female cabbage loopers in complete conformity with those of the natural pheromone.

The discovery that the male cabbage looper moth produces 2-phenylethanol is not surprizing, since this compound has previously been identified in males of *Mamestra configurata* Walker[21], *M. persicariae* (L.)[22], and *Polia nebulosa* (Hufnagel)[22], all members of the family Noctuidae, but from the scent brush organs of the 2nd sternite. This is the first time the compound has been shown conclusively to elicit precopulatory behavioural responses in any insect species. Although Gothilf and Shorey[10] were unable to determine the role of the male scent in the mating sequence of *T. ni*, the possibility still remains that 2-phenylethanol functions as an aphrodisiac by modifying the tendency of the female to accept the male; this possibility is under investigation. 2-Phenylethanol represents the first identification of a genital scent brush pheromone in the Noctuidae, and the first male pheromone identified in the subfamily Plusiinae.

Behavioural biossay tests conducted with serial dilutions of 2-phenylethanol on female loopers (Table) showed that the minimum amount needed to cause a strong sexual response was 1 µg; a weak response was obtained with 0.1 µg. At the 0.1 µg level, weak but significant antennogram and single cell responses were obtained (Table). This is in good agreement with the reported rise, at 0.1 µg, of the EAG dose-response curves

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for aromatic male pheromones in other noctuid species[18]. The fact that an average 16 µg of 2-phenylethanol was isolated from each male moth is a strong indication that the pheromone is stored in the body of *T. ni* in uncombined form. In *M. configurata*, it is stored as the hexoside and released in pure form, as needed, by enzymic hydrolysis.

In recordings from single olfactory receptors of the antenna of female *T. ni* conducted by one of us (E.P.), certain cells innervating sensory hairs (Sensilla trichodea) specifically responded to both 2-phenylethanol and male scent brush extract. Within the same type of sensillum, other receptor cells responded maximally to the corresponding aldehyde, phenylacetaldehyde. This compond has long been known[23,24] as a strong distance attractant for the female (and to a lesser degree, the male) cabbage looper, and was initially considered by us as the prime candidate structure for the male pheromone. However, no trace of phenylacetaldehyde could be detected in the scent brush extract, and in our behavioural bioassay this compound was totally inactive at all doses. Detailed reports will be given elsewhere on the responses of single pheromone receptor cells of the female *T. ni*, and on the chemical specificity of 2-phenylethanol determined by the novel laboratory behavioural bioassay described here.

HENDRICKS and SHAVER[25] have recently shown that an unidentified pheromone released from the hairpencils of another noctuid, the male *Heliothis virescens* (F.), prior to mating, suppresses the emission of sex pheromone by the female insect. Should this be true of 2-phenylethanol for *T. ni*, it might conceivably be used in controlling this pest through mating suppression. This interesting possibility is presently under investigation[26].

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[26] We thank Dr. P. A. GIANG for infrared spectra, Dr. N. WAKABAYASHI for gas chromatograms, Dr. R. M. WATERS for NMR-spectra, Dr. S. DUTKY for mass spectra, and Miss D. GOUGH for technical assistance in rearing the insects.

Jungermanool, a New Labdane Diol from the Liverwort, *Jungermannia torticalyx* Steph.[1]

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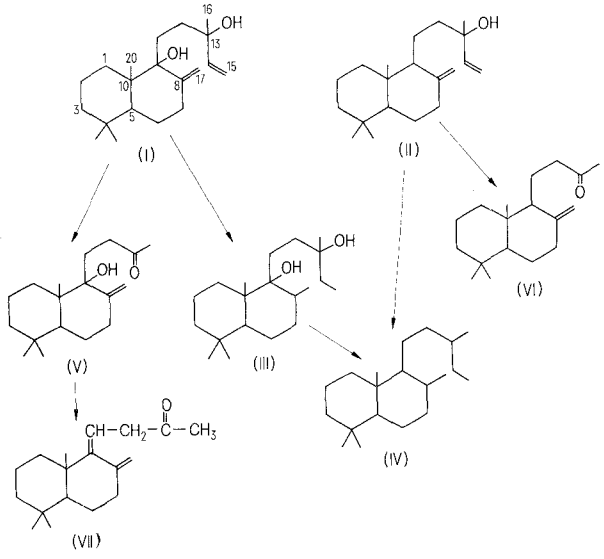
Summary. A new diterpenoid named jungermanool was isolated from *Jungermannia torticalyx* and the structure was found to be labda-8 (17), 14-dien-9, 13-diol by chemical and spectroscopical methods.

From the liverwort, *Jungermannia torticalyx* Steph., we isolated a new diterpene diol (I) named jungermanool together with (–)-manool[3], whose structure was determined to be represented by formula I.



Jungermanool (I), $C_{20}H_{34}O_2$ (M$^+$ 306); mp 124–125°; $[\alpha]_D$ –49.3°, was isolated from a hexane extract of the plant by elution chromatography. The IR- and PMR-spectra[4] resembled closely those of manool in the whole pattern. They exhibited the presence of 4 tertiary methyls (ν 1390, 1380, 1370 cm^{-1}; δ 0.81, 0.90, 1.00, 1.21, each 3H, s), 1 vinyl and 1 exomethylene (ν 3090, 1640, 987, 917, 880 cm^{-1}; δ 4.97, 1H, d.d, J=10.0, J=2.5; δ 5.15, 1H, d.d, J=15.0, J=2.5; δ 5.90, 1H, d.d, J=15.0, J=10.0; δ 4.52, 4.79, each 1H, br.s) as well as 2 tertiary hydroxyl groups (ν 3640, 3610, 3470 cm^{-1}). Its saturated tetrahydro derivative (III), $C_{20}H_{38}O_2$ (M$^+$ 310); mp 69–70°; $[\alpha]_D$ –26.9°; ν 3630, 3610, 3450 cm^{-1}, which was obtained by catalytic hydrogenation of the diol over PtO_2 in AcOH, after being dehydrated with $SOCl_2$ in pyridine, was submitted to catalytic hydrogenation over PtO_2 in AcOH to give labdane (IV),[5] $C_{20}H_{38}$ (M$^+$ 278); $[\alpha]_D$ –6.7°, which was identified by the coincidence of the IR-, PMR- and MS-spectra with those of an authentic sample prepared from manool. In addition, when the diol (I) was oxidized with $KMnO_4$ in acetone, a bisnor compound (V), $C_{18}H_{30}O_2$ (M$^+$ 278); mp 64–65°; $[\alpha]_D$ –55.0°;

[1] Chemical constituents from *Hepaticae*, Part XXIV: Part XXIII, A. MATSUO, H. NOZAKI, M. NAKAYAMA, Y. KUSHI, S. HAYASHI and N. KAMIJO, Tetrahedron Lett. *1975*, 241.

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