sophila to 67 in *Locusta*. Base exchanges occurred in an additional eight positions resulting in an overall homology of 87%.

Similar evolutionary processes can be deduced from the $tRNA^{ser}_{UCN}$ sequence. The *Locusta* gene of 70 nucleotides is 3 bp longer due to two base insertions in the TΨC and a third in the V loop. An additional seven base exchanges result in an overall homology of 86%. The $tRNA^{ser}_{UCN}$ sequence reveals an AA mismatch at the beginning of the anticodon stem (see arrow). An AA mismatch in the same position is reported for a tRNA Val gene in *Neurospora* [11]. Examples for mismatches in the TΨC stem are a TT mismatch in the $tRNA^{phe}_{UUC}$ gene of *Tetrahymena* [1] and in a tRNA Val gene from *A. albopictus* [5].

A copy of the $tRNA^{leu}_{CUN}$ gene indicated in Fig. 1A is found within a cloned DNA fragment of nuclear DNA homologous to mitochondrial genes [6, 12, 13] and is thus part of a "promiscuous, DNA" sequence.

We thank K. Weiler for typing the manuscript. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

Received November 26, 1986

1. Suyama, Y.: Nucl. Acids Res. *13*, 3273 (1985)
2. Anderson, S., et al.: Nature *290*, 457 (1981); J. Mol. Biol. *156*, 683 (1982); Bibb, M.J., et al.: Cell *26*, 167 (1981)
3. Clary, D.O., Wolstenholme, D.R.: J. Mol. Evol. *22*, 252 (1985)
4. HsuChen, C.-C., Kotin, R.M., Dubin, D.T.: Nucl. Acids Res. *12*, 7771 (1984)
5. Dubin, D.T., HsuChen, C.-C., Tillotson, L.E.: Curr. Genet. *10*, 701 (1986)
6. Gellissen, G., et al.: Nature *301*, 631 (1983)
7. McCracken, A., Uhlenbusch, I., Gellissen, G.: Curr. Genet. (in press)
8. Chomyn, A., et al.: Nature *314*, 592 (1985); Ise, W., Haiker, H., Weiss, H.: EMBO J. *4*, 2075 (1985)
9. Uhlenbusch, I., McCracken, A., Gellissen, G.: Curr. Genet. (in press)
10. Bastl, E., Dawid, I.B.: Cell *18*, 501 (1978); Wong, J.F.H., et al.: Nucl. Acids Res. *11*, 4977 (1983)
11. Heckman, I.E., et al.: Cell *13*, 83 (1978)
12. Gellissen, G., Michaelis, G.: Ann. N.Y. Acad Sci. (in press)
13. Gellissen, G.: BIUZ (in press)
14. Sanger, F., et al.: Proc. Nat. Acad. Sci. USA *74*, 5463 (1977)

Identification of 5,9-Dimethylheptadecane as a Sex Pheromone of the Moth *Leucoptera scitella*

W. Francke and S. Franke
Institut für Organische Chemie der Universität, D-2000 Hamburg

M. Toth and G. Szöcs
Research Institute of Plant Protection, H-1525 Budapest

P. Guerin and H. Arn
Swiss Federal Research Station, CH-8820 Wädenswil

With the exception of numerous oxygen-containing sex pheromones of moths, few hydrocarbons have been identified, most of which seem to be derived from straight-chain polyenic acids. Until now, only two branched hydrocarbons are known as sex pheromones of moths: 2-methylheptadecane, reported from several Arctiid species [1] and (*S*)-14-methyl-1-octadecene, found in the peach leafminer, *Lyonetia clerkella* L. [2]. From females of the mountain-ash bentwing, *Leucoptera scitella* (Zeller) (Lepidoptera: Lyonetidae), we have now identified 5,9-dimethylheptadecane, the first dimethyl-branched sex pheromone of moth species.

L. scitella occurs as a rather polyphagous pest in orchards all over the temperate regions of Europe and in some parts of Asia [3]. Due to favorable climatic changes it has become overwhelmingly important in Hungary during the past years, where it caused up to 50% loss of foliage in many commercial apple orchards on the plains [4]. The beginning of the flight period of this leafminer may shift from year to year for several weeks [5]. The use of synthetic pheromones in a monitoring system based on trap catches would be of enormous importance in the control of this pest. The objective of the present study was the identification and synthesis of behavior-mediating volatile compounds from the female-producd sex pheromone of *L. scitella*.

The insects used in our investigations emerged from overwintering pupae which had been collected in an apple orchard at Halásztelek, Pest County, Hungary. The moths were sexed immediately after emergence, and the sexes were held in separate chambers. For collection of the female sex pheromone 1–3-day-old females were used. Biologically active extracts were obtained by washing the abdominal tips of calling females in hexane. Volatiles released by calling females were trapped in a modified closed-loop stripping system [6]; on three different carbon filters a total amount of 600 female night equivalents (FE) were collected within three periods. The filters were extracted with small amounts of carbon disulfide, and the combined extracts were concentrated to 10 µl. Gas chromatographic analyses with electroantennographic detection (GC-EAD) using the male *L. scitella* antenna [7] were carried out on high-resolution GC columns (30 m, SE 30 and SP 2340) indicating the presence of an active non-polar component with a retention time very close to octadecane. For GC-MS analysis (Varian 311A instrument, EI, 70 eV) ca. 50 FE were injected on a 50 m Cp Sil 8 CB fused silica capillary column which was kept at 60 °C for 3 min, then programmed to 250 °C at 3 °C min^{-1}. Upon comparison with known mass spectra, small amounts of isopropyl tetradecanoate, octadecyl acetate, and the characteristic row of unbranched, uneven-numbered hydrocarbons C_{19}–C_{27}, typical for many insects, were easily identified. The main component in the head space extract showed a mass spectrum of a branched C_{19}-hydrocarbon; its retention time coincided with the EAD peak. Characteristic fragments in the spectrum [8] pointed to 5,9-dimethylheptadecane. A mixture of all possible four isomers was synthesized by Kolbe electrolysis: 2 g of racemic 3-methylheptanoic acid and 3 g of racemic 4-methyldodecanoic acid were dissolved in 50 ml methanol, neutralized with trimethylamine and submitted to electrolysis for 2 h at room temperature. Pt electrodes were used at a current density of 0.3 A cm^{-2}. Usual work-up yielded 30% of the desired

Table 1. Captures of male *L. scitella* by different amounts of 5,9-dimethylheptadecane (Halásztelek, Pest County, Hungary, August 12–21, 1985)

Dosage [μg]	1	2	3	Total[a]
1000	147	86	98	331a
100	23	31	21	75ab
10	0	5	26	33bc
1	0	1	9	10c
unbaited	5	0	0	5c

[a] Captures with same letter are not significantly different at $P=5\%$ by Duncan's NMRT after $\log(x+1)$ transformation of data

product (bp[1] 83 °C) which was further purified by preparative GLC on SE 30. The method described here provides an excellent tool for the synthesis of chiral-branched hydrocarbons starting from optically active branched carboxylic acids.

The diastereomers of 5,9-dimethylheptadecane could not be separated by GC; however, retention time and mass spectrum matched the data of the natural product. Results of field tests with the synthetic product are compiled in Table 1. The data clearly substantiate the high biological activity of 5,9-dimethylheptadecane. The syntheses of all possible four optically pure isomers of 5,9-dimethylheptadecane and results concerning their biological activity will be published separately.

The new pheromone 5,9-dimethylheptadecane belongs to a widespread type of compounds showing an odd number of methylene interruptions between two methyl branchings. High-boiling hydrocarbons with this pattern have been identified from the cuticular lipids of flies, ants, beetles, cockroaches, and locusts [9]. Similar compounds like 15,19,23-trimethylheptatriacontane are known as sex pheromones of tse-tse flies, *Glossina* spp [10]. In contrast to the common unbranched pheromones which originate from the acetate pool, the methyl-branched compounds mentioned above indicate mixed biosyntheses from propionate (methylmalonate) and acetate units. "Insertion" of two subsequent propionate units into a chain would yield 1,3-dimethyl branchings, while propionate-acetate-propionate leads to 1,5-dimethyl branchings (see 5,9-dimethylheptadecane) – an additional acetate unit will separate the methyl groups to 1,7-dimethyl branching etc. Homomevalonate may also be involved in the biogeneses of some of the compounds.

This research was supported by the Fonds der chemischen Industrie and the Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung. We express appreciation to Dr. G.Y. Sziráki, Budapest, for supply of the insects.

Received September 23
and November 3, 1986

1. Roelofs, W.L., et al.: Science *171*, 684 (1971)
2. Sato, R., et al.: Appl. entomol. Zool. *20*, 411 (1985)
3. Réal, P., in: Entomologise Appliquée à l'Agriculture II, p. 256 (A.S. Balachowsky, ed.). Paris: Masson 1966; Briolini, G.: Boll. Inst. Ent. Univ. Bologna *24*, 239 (1960); Ferro, S.: Boll. Lab. Ent. Agr. Portici *19*, 53 (1961); Dulic, K., Unjac, M.: Zast. Bilja *32*, 409 (1981); Ivanov, S.J.: RAst. Zasch. Sofia *9–10*, 27 (1967)
4. Molnár, J.: Növényvédelem *18*, 350 (1982); Dulinafka, Gy.: ibid. *19*, 155 (1983)
5. Balász, K.: ibid. *19*, 305 (1983)
6. Boland, W., et al., in: Analysis of Volatiles, p. 371 (P. Schreier, ed.). Berlin–New York: de Gruyter 1984
7. Arn, H., et al.: Z. Naturforsch. *30c*, 722 (1975)
8. Pomonis, J.G., et al.: J. chem. Ecol. *6*, 965 (1980)
9. Howard, R.W., Blomquist, G.J.: Ann. Rev. Entomol. *27*, 149 (1982); Genin, E., et al.: J. chem. Ecol. *12*, 1213 (1986)
10. Carlson, D.A., et al.: Science *201*, 750 (1978)

In Vitro Effectiveness of a Mistletoe Preparation on Cytostatic-Drug-Resistant Human Leukemia Cells

H. Hülsen and F. Mechelke
Institut für Genetik der Universität Hohenheim, D-7000 Stuttgart

Preparations of *Viscum album* L. (European mistletoe) have been used against a variety of diseases, such as arthrosis, rheumatism, hypertension [2], and also in the treatment of human cancer [2]. In our laboratory we have investigated the effectiveness of a mistletoe preparation, trade name HELIXOR®, on in vitro cell cultures of several tumor cell lines [3, 4, 8], especially the human leukemia cell line Molt 4 [3, 4] which was derived from a 19-year-old male patient with acute lymphoblastic leukemia of T-cell type [5]. Acute lymphoblastic leukemia (ALL), a cancerous transformation of lymphocyte stem cells in the bone marrow, is one of the main cancerous diseases of children and young adults. By means of chemotherapy with cytostatic drugs together with radiotherapy, modern medicine is today able to ob-

Table 1. In vitro sensitivity and resistance against cytarabine and methotrexate of the human leukemia cell line Molt 4 and of three drug-resistant sublines, indicated as LD_{50}-values and resistance factors. The LD_{50} is the drug concentration, in which the cell cultures contain half as many living cells as the untreated (control) cultures after 72 h treatment with the individual drugs. The resistance factors are calculated by comparing the LD_{50} of the original Molt 4 cells and of the drug-resistant sublines. This table shows also the sensitivity of all four cell lines against the mistletoe preparation HELIXOR M. The LD_{50}-values of HELIXOR M indicate that the drug-resistant sublines show a retaining or even an increasing sensitivity against HELIXOR M

Cell line	Cytarabine [μg/ml]	Methotrexate [μg/ml]	Resistance factor	HELIXOR M [μg/ml]
Molt 4	0.002	0.015	–	82.2
M4-MTX^r-1	–	72.6	4,840	47.9
M4-MTX^r-2	–	16.6	1,107	36.8
M4-Ara-C^r-1	4.6	–	2,300	80.9

