Naturwissenschaften 78, 521 – 523 (1991)

(*R,Z*)-5-(−)-(Oct-1-enyl)oxacyclopentan-2-one, the Sex Pheromone of the Scarab Beetle *Anomala cuprea*

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The cupreous chafer beetle *Anomala cuprea* Hope (Coleoptera: Scarabaeidae), *douganebuibui* in Japanese, is one of the most severe agricultural pests in Japan, which attacks a wide variety of crops both in the larval and adult stages. Because of the demand for safer agrochemicals (and the difficulty to control the pest with insecticides), the elucidation of behavior-modifying chemicals of potential use in pest management of the beetle is of utmost importance. However, pheromone identification in scarab beetles has been restricted to only a few species, mainly the Japanese beetle *Popillia japonica* [1] and the soybean beetle *Anomala rufocuprea* [2]. Preliminary works with the cupreous chafer were unrewarding [3], probably due to the complicated communication system of the insect. Furthermore, it has been considered that this insect does not use sex pheromones because of its gregarious nature. In fact, *A. cuprea* possesses a sex pheromone, whose full identification as (*R*, *Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one (*1*) is described here (Fig. 1).

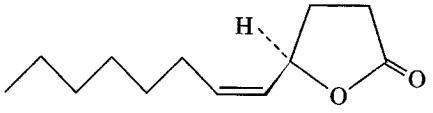

Fig. 1. Structure of *1*

The airborne volatiles of field-captured female beetles were collected on Tenax (airflow rate of 2.5 l/min), extracted with hexane, concentrated to 0.1 female equivalent (FE) per μl and bioassayed. When a 5 FE containing filter paper (FP) was set on a 25 × 35 cm plastic box, 10 out of 16 males walked towards the FP and/or tried to copulate with each other by holding another individual by his back and displaying the penis.

The GC generated by these extracts was highly contaminated by volatiles of the foodstuff when an artificial diet for wild silk moth (Nihon Chlorella Co., Ltd.) was used during aeration, but it was less contaminated when the insects were fed on grape leaves. This was separated in an SiO_2 column by successively eluting whith a stepwise hexane-ether mixture. Screening of all fractions revealed that only the 20% ether containing fraction was biologically active. By coupling GC with an electroantennographic detector, GC-EAD [4], and using male antennae, only one active peak appeared from the crude extract at t_R 23.6 min (30 m × 0.254 mm fused-silica column coated with 0.25 μm of DB-23 stationary phase), which was recovered solely in the 20% fraction. No fractions generate an EAD peak when female antennae were used [5]. These results and the fact that extracts obtained by aeration of male beetles did not contain the same compound strongly suggested that a peak at t_R 23.6 min was the sex pheromone of this beetle.

Electron-impact mass spectrum (EI-MS) of the active peak gave the molecular ion at m/z 196 (7%) and the base peak at m/z 111. Other fragments were: 41(48%), 55(37), 67(32), 81(41), 98(22), 126(22), 136(17), 153(7), 167(3), and 181(0.6). That the molecular peak was m/z 196 was corroborated by chemical ionization with NH_3 (214, base peak) and CH_4 (237 [M + 41], 214 [M + 18], 197 $[M + H]^+$, 179 $[M + H]^+ - H_2O$, base peak, 161 and 137). Both the precise MS (m/z 196.1451) and atomic emission detector measurements supported the formula $C_{12}H_{20}O_2$ (which requires 196.1463). Vapor phase IR (Fig. 2) gave a strong band at 1811 cm^{-1} (ν C=O), along with signals at 1157 [νC-C(=O)-O] and 1027 (νO-C-C), which was considered to be due to a γ-lactone. That the pheromone possessed a double bond (with cis-configuration) was indicated by the signal at 3020 cm^{-1}. As the predominant mass fragmentation process of simple γ-lactones, leading to the characteristic ion at m/z 85 was suppressed, the double bond was assumed to be adjacent to the ring, which would give rise to the base peak at m/z 111 [6]. Thus, the molecular structure was temporarily assigned as (?,*Z*)-5-(oct-1-enyl)oxacyclopentan-2-one; its absolute configuration, which was achieved by the syntheses of both enantiomers and chiral GC measurements, remained to be determinded.

The procedures for the racemic and asymmetric syntheses are outlined in Fig. 3. Coupling of 1-octyne with methyl 4-oxobutyrate gave, after heating, the corresponding acetylenic lactone, which was reduced with a Lindlar catalyst to yield the racemic pheromone. It gave a single peak (both on DB-23 and DB-1) of MS and t_R identical to those of the natural products. Optical resolution was achieved with a Chiraldex G-TA (ASTEC) 20 m × 0.25 mm I.D., 0.125 μm column operated at 120 °C (head pressure 2 kg/cm^2). Surprisingly, the enantiomer retention times (Fig. 4) differed for over 7 min (the identity of the peaks was confirmed by GC-MS). In order to determine the absolute configuration of the resolved peaks, an enantiomeric mixture was prepared via an ap-

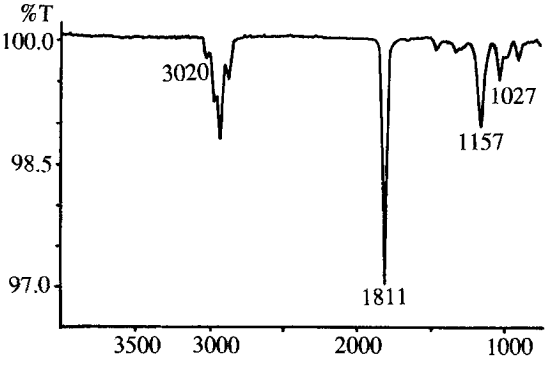

Fig. 2. Vapor-phase infrared spectrum of *A. cuprea* sex pheromone *1*

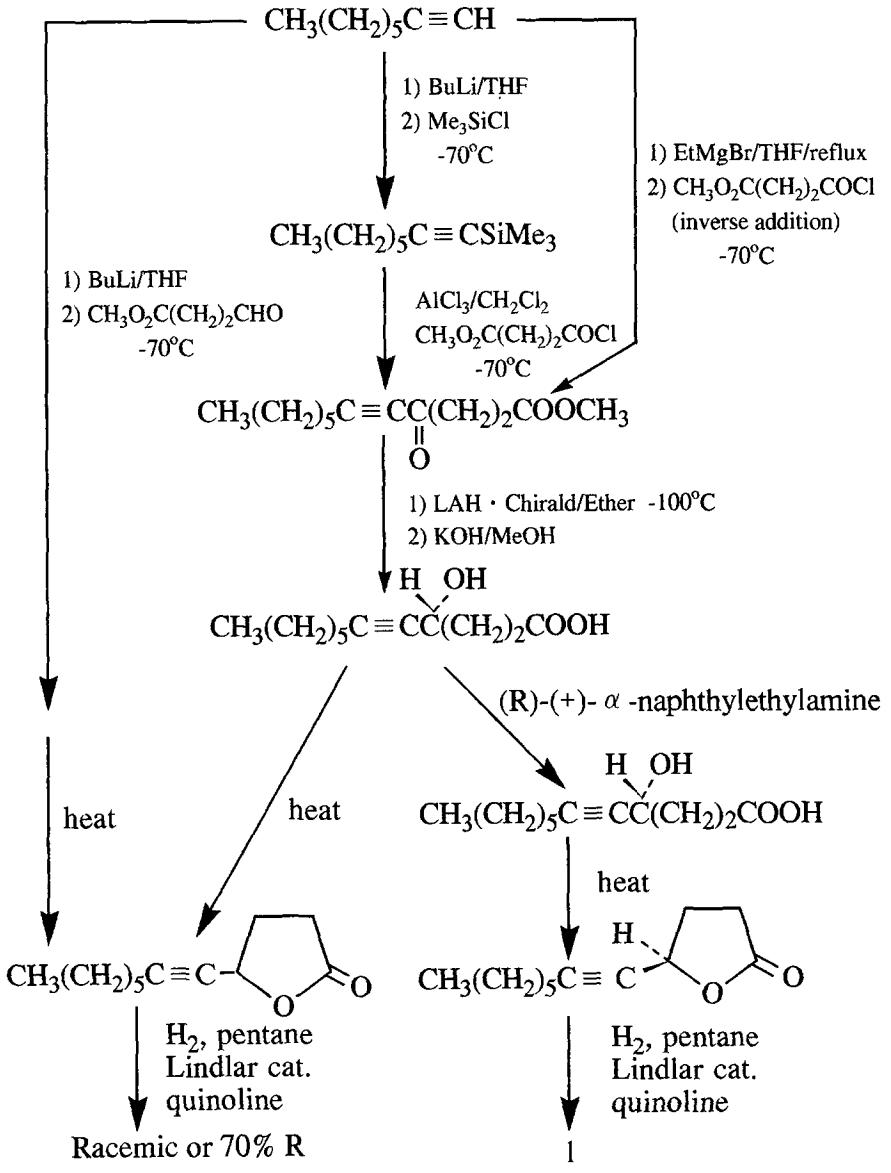

Fig. 3. Syntheses of the enantiomers of the pheromone

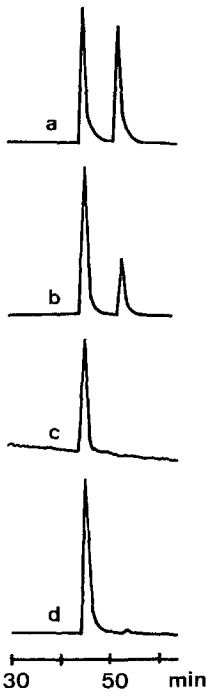

Fig. 4. Enantiomeric resolution of (*Z*)-5-(oct-1-enyl)oxacyclopentan-2-one, a) racemic, b) *R*-rich enantiomeric mixture, c) natural product, d) synthetic pheromone

propriate acetylenic ketone, whose asymmetric reduction with Chirald is known to give predominantly the corresponding carbinol with the *R*-configuration [7, 8]. Thus, after lactonization and reduction a mixture rich in the *R*-enantiomer could be obtained.

Acylation of 1-trimethylsilyl-1-octyne with 3-carbomethoxypropionyl chloride in the presence of aluminum chloride or by Grignard reaction starting from 1-octyne (Fig. 3) gave the required propargyl ketone, methyl 4-oxo-dodec-5-ynoate [IR 1744 cm^{-1}, $\nu C(=O)OMe$, 1680, $\nu C=O$, and 2212, $\nu C\equiv C$; EI-MS m/z 137 (100%), m/z 193(6); CI-MS $[M + NH_4^+]$ 242; NMR 90 MHz ($CDCl_3$) δ 0.9 (3H, distorted), 1.3 (8H, br), 2.4 (2H, t, J = 6.6 Hz), 2.5–3 (4H, m), and 3.7 (3H, s)]. Its asymmetric reduction gave methyl 4-hydroxy-dodec-5-ynoate [IR 1738 cm^{-1}, $\nu C(=O)OMe$, 2242, $\nu C\equiv C$, and 3486 (br), νOH; NMR 0.89 (3H, distorted t), 1.28 (8H, br), 1.8–3 (7H, m), 3.69 (3H, s)]; by using $Eu(ftc)_3$ [8], the signal at 3.69 was split into two (3.887 and 3.816). Thus the optical purity was determined to be ca. 75%. Hydrolysis gave the corresponding γ-hydroxy acid which, on distillation, yielded (*R*)- and (*S*)-5-(oct-1-ynyl)oxacyclopentan-2-one [IR 1785 cm^{-1}, $\nu C=O$, and 2242, $\nu C\equiv C$; EI-MS 85 (100%), 79(91), 165(52), and 179(1); CI-MS $[M + NH_4]^+$ 212; NMR 0.82 (3H, distorted t), 1.28 (8H, br), 1.9–2.8 (6H, m), 5.1 (1H, br)]. Semihydrogenation with Lindlar catalyst in pentane in the presence of quinoline gave an enantiomeric mixture of 70% (*R*,*Z*)- and 30% (*S*,*Z*)-5-(oct-1-enyl)oxacyclopentan-2-one, as revealed by GC (Fig. 2).

The natural pheromone gave a single peak corresponding to the (*R*, *Z*)-configuration (Fig. 4). Therefore, the sex pheromone was fully assigned as (*R*, *Z*)-(−)-5-(oct-1-enyl)oxacyclopentan-2-one, which is novel both synthetically and in nature.

Optically pure pheromone was prepared by using a resolving reagent previously used in the syntheses of sex pheromones, namely, (*R*)-α-(+)-napthylethylamine [8, 9] to further enrich the desired enantiomer, (*R*)-4-hydroxy-dodec-5-ynoic by recrystallization. Acidification of the pure salt, followed by distillation gave the *R*-acetylenic lactone, which was reduced as before to yield (*R*,*Z*)-5-(−)-(oct-1-enyl)oxacyclopentan-2-one [IR 1780, 1178, 1016, and 3017 cm^{-1}; NMR 0.86 (3H, dis-

tortedt), 1.28 (8H, br), 1.6 – 2.7 (6H, m), 4.8 – 5.8 (3H, m); $[\alpha]_D^{24}$ – 61.2° (*c* 0.85, hexane)]. As determined by chiral GC (Fig. 4), the optical purity of the pheromone was 98.4%, corresponding to an enantiomeric excess of 97%.

The amount of material collected on the aeration apparatus (which gave a recovery ratio of 87% for methyl myristate as a standard at the 100 ng level in 4 h) from field-captured female beetles was on average less than 150 ng/female a day. Nevertheless, an experiment with four virgin females (kindly provided by M. Hasegawa of Chiba Prefectural Agricultural Experiment Station) gave as much as 2000 ng per female and day. This discrepancy might be due to the decrease in the release of pheromone with the age of the captured insects. By and large, sex pheromones are released at a much lower level, but higher rates have been reported even in lepidopterous insects [10].

The pheromonal activity of the synthetic enatiomer was demonstrated by two bioassays. Using the same procedure for screening the natural product, 100 ng of synthetic pheromone was transferred to FP. When submitted to three groups of 10 male beetles, 80% of the insects elicited sexual behavior. In all the ranges tested, namely, from 0.05 to 100 FE, activity was displayed. The attractancy of the pheromone was tested in a wind tunnel (2 m long, 30 cm I.D.) internally covered with wire mesh in order to allow the insects to walk in a similar manner to that in the bioassay for the soybean beetle [11]. Groups of ten insects were placed at the downwind end of the tunnel and observed for 15 min at an airflow of 40 cm/s, 1 lx, and 24°C. In the control, only one or two individuals moved upwind, but when samples of synthetic pheromone were set in an FP 1.5 m away from the insects, they extended their antennae, opening the plates, became very excited, walked towards the FP, visited the pheromone source many times, and tried to fly, but were prevented by the narrow space. In the range tested (1 – 100 FE), the greater the amount of pheromone, the higher the response was; in a typical experiment with 100 FE of synthetic pheromone all the individuals visited the pheromone source within 15 min.

Although these findings contribute a great deal to the understanding of cupreous chafer pheromonal chemistry, more studies on the behavior of the insect are needed to guarantee the effective use of its pheromone in pest management.

I thank Dr. S. Wakamura for his suggestion to undertake this work, F. Mochizuki for his help in building the wind tunnel, Dr. M. Tsukada for the IR measurements, and Dr. T. Suzuki, Tsukuba University, for the measurements of optical rotation.

Received July 23 and August 16, 1991

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Naturwissenschaften 78, 523 – 526 (1991)

Worker-Brood Genetic Relatedness in a Primitively Eusocial Wasp

A Pedigree Analysis

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A striking feature of eusocial insects is the differentiation of colony members into a fertile reproductive caste and a sterile worker caste [1, 2]. High worker-brood genetic relatedness is the most widely accepted explanation for the apparent altruism on the part of workers that is implied by such reproductive caste differentiation. The haplodiploid genetic system leads to high genetic relatedness between full sisters (r = 0.75) in the Hymenoptera, an insect order with multiple origins of eusociality [1, 3]. However, polyandry (multiple mating by queens) and polygyny (the simultaneous presence of more than one queen in a colony) reduce worker-brood genetic relatedness [4]. Here, we show, by pedigree analysis in a primitively eusocial wasp, that even when there is only one queen at any given time, serial polygyny (the frequent replacement of queens) leads by itself, and even more potently in combination with polyandry, to a substantial reduction in worker-brood genetic relatedness.

Nests of the primitively eusocial wasp *Ropalidia marginata* may be initiated by one or a small number of females [5, 6]. In multiple foundress nests, only one female becomes the egg layer or queen while others assume the role of workers. Female offspring may either leave their natal nests to found new single- or multiple-foundress nests or may remain and assume the role of