Agric. Biol. Chem., 53 (11), 3057~3059, 1989

Note

Isolation and Identification of a Kairomone Responsible for the Stinging Behavior of *Bracon hebetor* Say (Hymenoptera: Braconidae) from Frass of the Almond Moth *Cadva cautella* Walker

Jun-ichi FUKUSHIMA, Yasumasa KUWAHARA and Takahisa SUZUKI

Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Received May 8. 1989

Bracon hebetor Say is a cosmopolitan parasitoid wasp that hunts phycitid moth larvae as its hosts.[1] Females paralyze the host larvae,[2,3] not only to lay eggs on them for raising their offspring, but also to feed on larval hemolymph to enable their ovalioles to mature.[4,5] When the female wasp locates a host larva (*Cadra cautella* Walker in this study), she examines the larva with antennal tapping, and then bends her abdomen to sting the host for anesthesia. We now report the isolation and identification of the kairomone(s) responsible for eliciting this sequence of characteristic behavior from the frass of host larvae.

The hexane extract of the frass indicated kairomone activity, displaying the stinging behavior within 10 min, when a piece of filter paper (5 mm x 5 mm) impregnated with the extract was introduced to each of 10 glass petri dishes (15 mm id × 10 mm, Mini-p), a mated and starved female of 3 days old being kept in each. By monitoring the kairomone activity by the sequetial host location and abdominal bending behavior, the kairomone was isolated from 7.2 kg of frass.

The hexane extract (342 g) of the frass (7.2 kg) from the almond moth *Cadra cautella*, after evaporating of the solvent, was chromatographed 3 times on a SiO_2 column (total 3 kg, 55 cm × 8 cm i.d.), and the column was eluted with 5 l each of following solvents: hexane. 10% and 50% ether in hexane. ether and methanol. The activity was located in the ether and methanol fractions, from which an acidic and non-urea-includible fraction (3.7 g) was collected. This fraction, after purifying through a Sephadex LH-20 column (35 g, 50% CHCI, in MeOH), was methylated with diazomethane. The resulting methylated kairomone (M-kairomone) was purified by an SiO_2 column (20 g, 24 cm × 1.4 cm i.d.). The M-kairomone fraction was recovered in 30% ether in hexane. This fraction was again chromatogrphed by an SiO_2 column to give pure M-kairomone (254 mg; one peak by GLC on 5% OV-I. t_R 3.27 min). Thecompound indicated activity at 2×10^{-3} μg after hydrolysis, while the crude hexane extract was active at 2×10^{-2} μg (Table I). Therefore some other inactive fractions by themselves might have synergized or enhanced the activity.

^{1}H-NMR spectrum (100 MHz) of M-kairomone displayed the following chemical shifts: 6 0.9 (t), 1.3 (br. s). 2.1 (m), 2.3 (m). 3.5 (m), 3:8 (s) and 5.5 (m), indicative of methyl hydroxy alkenoate. The presence of ester (1735 and 1070 cm^{-1}) and of hydroxy group(s) (3400 cm^{-1}), and non-presence of an E geometry of the double bond (*ca.* 970 cm^{-1}) were also supported by IR. No information about the M^+ ion was obtained by GC/MS of M-kairomone, apart from the following fragment ions: *m/z* 280. 185 and 156. The acetylated M-kairomone (MA-kairomone) was then subjected to GC/MS (Fig. I). to give characteristic fragment ions at *m/z* 381 and 352, both of which corresponded lo $M'-31$ and M^+-60, respectively. As a result. the M^+ ion was concluded to be *m/z* 412 (not detectable). suggestive of methyl diacetoxy-substituted octadecenoate.

High-resolution 'H-NMR (500 MHz) of the MA-kairomone (Fig. 2) gave two acetoxy methyls at δ 2.08 and 2.06, and two kinds of $-CH_2-$ (adjacent to COOMe and inbetween CH–OAc and C=C) at 6 2.3. Almost the same chemical shifts (6 2.08 and 2.06) of two acetyis and their relatively lower shift suggested a 1,2-diacetoxy structure (or before acetylation, 1,2-dihydroxy moiety). The double bond configuration was assigned as *cis*, based on the coupling constant (10.8 Hz) of the vinyl protons.

Since the M-kairomone gave 3-nonenal and methyl 8-formyloctanoate by oxidative glycol fission. two hydroxy groups were substituted at the 9th and 10th carbon of methyl octadecenoate, and the double bond was present inbetween the 12th and 13th carbon. The dimethyldisulfide adduct of MA-kairomone gave the molecular ion at *m/z* 506, and characteristic ions at *m/z* 475 (M^+-31). 315 and 131, which also supported the structure unambiguously (Fig. 1).

Dihydroxylation of linoleic acid with performic acid led to a diastereomer (*S,R* and *R,S*), while permanganate led to the other (*S,S* and *R,R*). Vinyl protons of the former appeared at δ 5.482 and 5.292 (10.8 Hz, d, 7.3 Hz, t, 1.8 Hz,

Table I. MINIMUM AMOUNTS OF COMPOUNDS REQUIRED TO DEVELOP KAIROMONE ACTIVITY

Crude hexane extract	2×10^{-2} μg
Natural kairomone	2×10^{-3} μg
Preparative kairomone (*S,S* and *R,R*)	2×10^{-2} μg
Preparative kairomone (*S,R* and *R,S*)	2×10^{-2} μg
Linoleic acid	$>2 \times 10$ μg

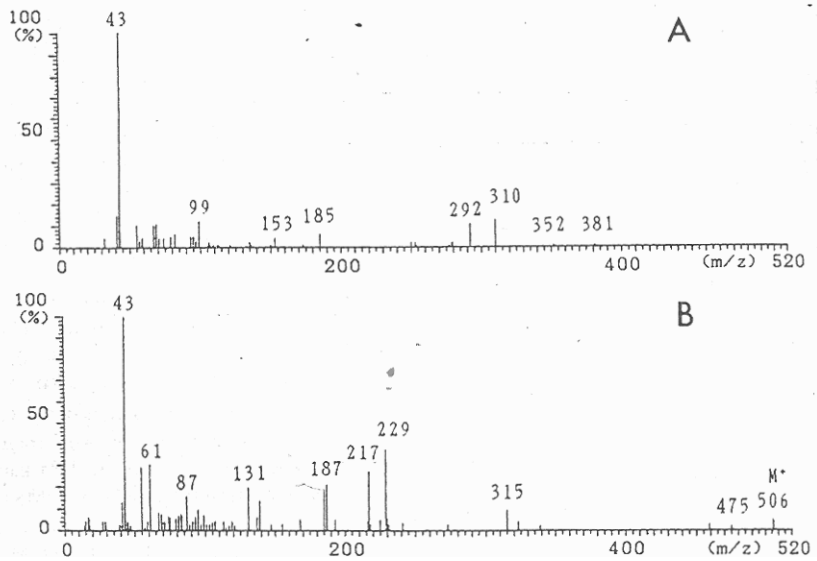

Fig. I. Mars Spectra of the Kairomone Derivatives.
A. methyl acetyl kairomone; B. DMDS adduct of methyl acetyl kairomone.

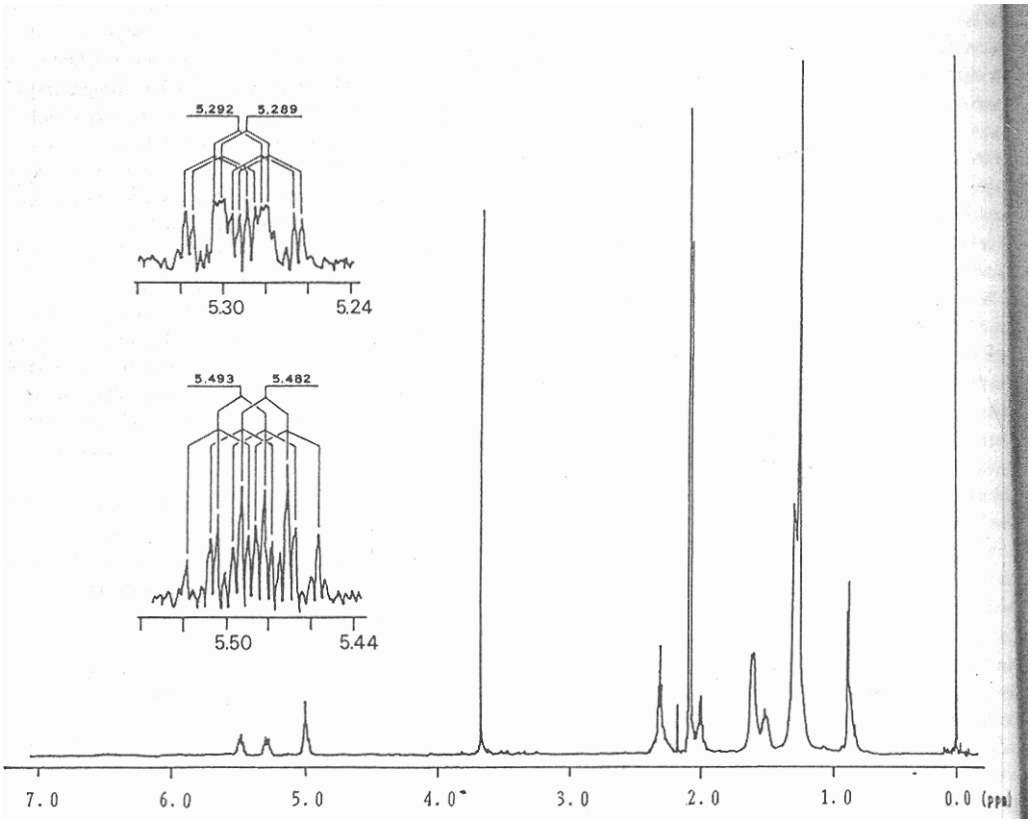

Fig. 2. High-resolution NMR Spectrum of Methyl Acetyl Kairomone.

t), and of the latter at 5.493 and 5.289. Natural MA-kairomone contained all the sets of vinyl protons (Fig. 2), suggestive of a mixture of both diastereomers, although natural M-kairomone indicated optical activity at $[\alpha]_D^{21}$ +2.09° ($c=4$, in MeOH).

From the results, the structure of the kairomone was concluded to be 9,10-dihydroxy-*cis*-12-octadecenoic acid with four possible stereoisomers. This compound is known as a plant growth regulator?' but has not previously been reported from the animal kingdom.

Although bath diastereomers after hydrolysis indicated the same kairomone activity at 2×10^{-2} μg, the activity reone-tenth that of the isolated kairomone, indicating some stereochemical requirements for developing the activity. Further studies on the stereochemistry of the kairomone and of the co-factors synergizing with 'the kairomone will be discussed elsewhere.

Acknowledgments. This work was supported in part by a grant for scientific research (No. 61560132) from Ministry of Education, Science and Culture of Japan. The authors thank Mr. Shigeki Yamada far his technical assistance at the beginning of this study.

References

1) J. W. Press and B. R. Flaherty, J. Go. *Entomol. Ecol.,* ***16.*** 342 (1980).
2) A. D. Taylor, J. *Anim. Ecol.*, ***57.*** 173 (1988).
3) D. W. Hagstrum, *Environ. Entomol.*, **12**, 1727 (1983).
4) R. M. Petters and D. S. Grousch, ***Ann.*** *Entomol. Soc.* ***Am.,*** **70**, *577* (1977).
5) D. W. Hagstrum and B. J. Smittle, *Environ. Entomol.*, ***7.*** 596 (1978).
6) H. Fukui, K. Koshimizu, S. Usuda and Y. Yamazaki *Agric. Biol. Chem., 41,* 175 (1977).