The Scent Substances of Pierid Butterflies (*Hebomoia glaucippe* Linnaeus) and the Volatile Components of Their Food Plants (*Crataeva religiosa* Forst.)

Nanao Hayashi, Akihiko Nishi, Tadayuki Murakami, Kazuyuki Maeshima, and Hisashi Komae

Study of Environmental Science, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730, Japan

and Takashi Sakao

Department of Chemistry, Faculty of Education, Kagoshima University, Kagoshima 890, Japan

Z. Naturforsch. **40 c**, 47–50 (1985); received August 14/October 24, 1984

Hebomoia glaucippe, Pierid Butterfly, Scent Secretion, E-β-Ocimene, *Crataeva religiosa*, Essential Oil

The scent secretions of two subspecies of *Hebomoia glaucippe*, which are pierid butterflies, and the essential oils of their food plant were investigated by means of GC and GC-MS. The scent substances of either subspecies are found to be only *E*-β-ocimene, and the volatile components of *Crataeva religiosa* were identified as 2-hexanal, 3-hexen-1-ol, *p*-cymene, limonene, linalool, α-ionone, and β-ionone. Electroantennogram (EAG) were recorded with the antennae of adult male and female butterflies. It was found that EAG responses of male to *E*-β-ocimene were greater than those of females.

Introduction

Hebomoia glaucippe Linnaeus distributed in Southeast Asia is the largest butterflies in the family, Pieridae. Some of the pierid butterflies are known to contain scent substances in their scent organs. Chemical studies on the scent secretions of several pierid butterflies such as *Pieris brassicae*, *Pieris rapae* and *Pieris napi* [1], *pieris napi japonica* Shirozu [2], and *Pieris melete* Menetries [2, 3] have been reported and some monoterpenes were identified. Meanwhile, it has been known that *Crataeva religiosa* Forst. (Capparidaceae) distributed in tropical and subtropical region and having characteristic odour is the food plant of *H. glaucippe*. In the present work, we examined the constituents of the scent secretions in the scent scale of the wings and other portions of *H. glaucippe liukiuensis* Fruhstorfer and *H. glaucippe formosana* Fruhstorfer collected in Japan and Formosa respectively, and the volatile constituents of *Crataeva religiosa* with interest for biochemical and/or biological connections between butterflies and their food plants. In addition, to clarify the biological activity of the scent secretions, electroantennogram (EAG) responses of the adult both sexes of the butterflies to the scent substance were also examined.

Reprint requests to Prof. T. Sakao.

0341-0382/85/0100-0047 $ 01.30/0

Experimental

Insects

Adults of both sexes of *Hebomoia glaucippe formosana* and *H. glaucippe liukiuensis* were captured during March in Formosa (Kenting) and during July in Japan (Setouchi cho in Amami-ōshima), respectively. The number of males and females used for this study were 20 respectively.

Collection of the secretion

When the butterflies were captured, the wings were amputated immediately, and the wings and bodies (head, thorax, abdomen were treated together) were extracted separately for 24 h at room temperature with 3 ml of dichloromethane. The extracts of the male and female were concentrated to 10 µl under a nitrogen stream at room temper-

ature. Each 1 µl portion of the extracts were injected into gas chromatograph (GC) and gas chromatograph-mass spectrometer (GC-MS).

Catalytic hydrogenation of the extracts of the butterflies

A portion (5 µl) of the extract was hydrogenated with PtO_2 catalyst (8 mg) over acetic acid (2 ml) for 3 h. The catalyst was filtered off and water was added to the filtrates to extract the hydrogenation product with ethyl ether. Evaporation of the solvent gave the hydrogenation product which was analyzed by GC and GC-MS.

Isolation of the essential oil

The fresh leaves (200 g) of *Crataeva religiosa* were collected at Setouchi Cho in Amami-ōshima, Japan, and chopped up, the cut materials were steam-distilled. The essential oil (0.1 g) obtained by the extraction with ethyl ether was analyzed by GC and GC-MS.

Chemical analyzes

Analyzes by GC were carried out with a Gasukuro Kogyo model 350 which was combined with an electric integrator and equipped with a flame ionization detector on OV-101 glass capillary column (0.28 mm × 40 m) programmed from 80° to 200 °C at 2 °C/min. The quantity of *E*-β-ocimene in the wings and bodies were determined by comparing the integration of their peak area on GC with those of reference standard solutions of *E*-β-ocimene. Analyzes by GC-MS were conducted with a JEOL JGC-20KP Gas Chromatograph and a JEOL JMS D-100 Mass Spectrometer. Analytical condition were as follows: column temperature, from 60° to 200° (8 °C/min); injection temperature, 200 °C; ionization voltage, 25 eV.

EAG recording

Two silverhalide coated wires in a glass capillary filled with Ringer's solution were inserted into the scape and apical part of the antenna, respectively. Both electrodes were connected to an amplifier. The amplified signal was recorded by an X-Y recorder. A filter paper disk (1 cm in diameter) impregnate with a test chemical was placed in 5 ml syringe. After 4.5 ml of air had been drawn into syringe, the odour was mixed into the air stream (3 ml/min) by pushing the plunger, so that the air including the odour was puffed onto the detached antenna through a 0.5 cm diameter glass tube.

Results and Discussion

Identification of E-β-ocimene as the scent substance of Hebomoia glaucippe

As shown in Fig. 1 (A), gas chromatogram of the scent substance of any portions of the butterflies revealed only one peak (R_t 15.7). By mass spectrometry, the peak gave the molecular ion at *m/z* 136 (in Fig. 2) and all of the fragmentation patterns were identical to those of the authentic *E*-β-ocimene [4]. The retention time on gas chromatogram (in Fig. 1 (B)) and the mass spectra (M^+ 142 in Fig. 3) of the hydrogenation product also agreed with those of hexahydroocimene.

Both *H. glaucippe liukiuensis* in Amami-ōshima and *H. glaucippe formosana* in Formosa contained only *E*-β-ocimene as the odour component and no other components were detected on the wings and bodies. The adult male and female butterflies emerged from pupae in the laboratory have the same scent odour (*E*-β-ocimene only) in the body and wings as the butterflies caught in the field.

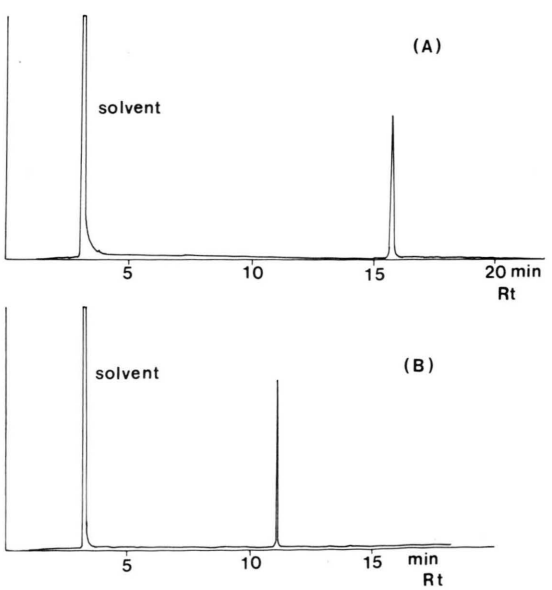
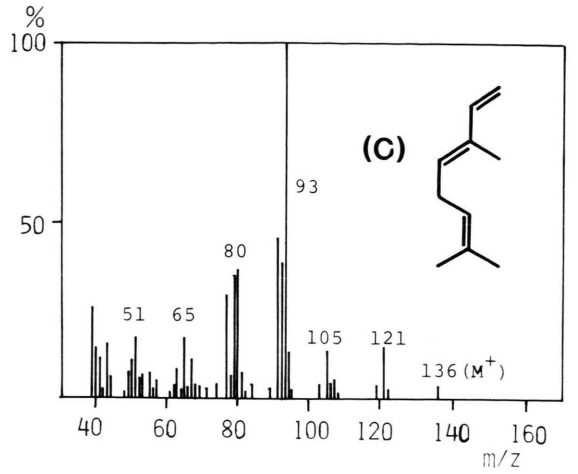


Fig. 1. Gas chromatogram of the scent secretion of male wings of *Hebomoia glaucippe liukiuensis* (A) and its hydrogenation product (B).



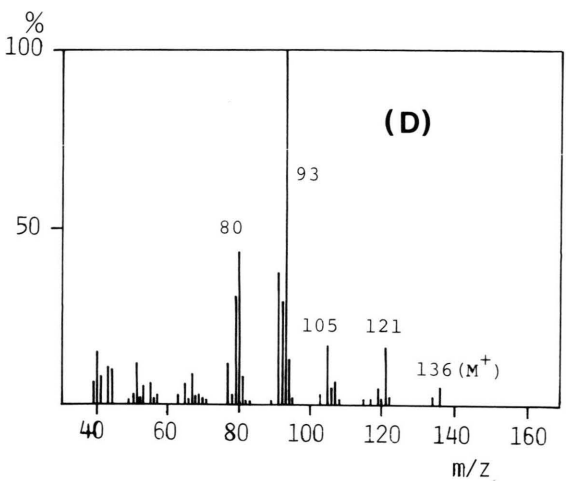
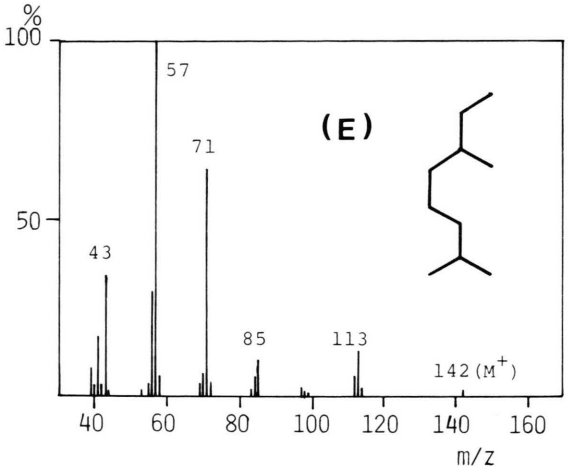


Fig. 2. Mass spectra of authentic *E*-β-ocimene (C) and scent substance of male wings of *Hebomoia glaucippe liukiuensis* (B).



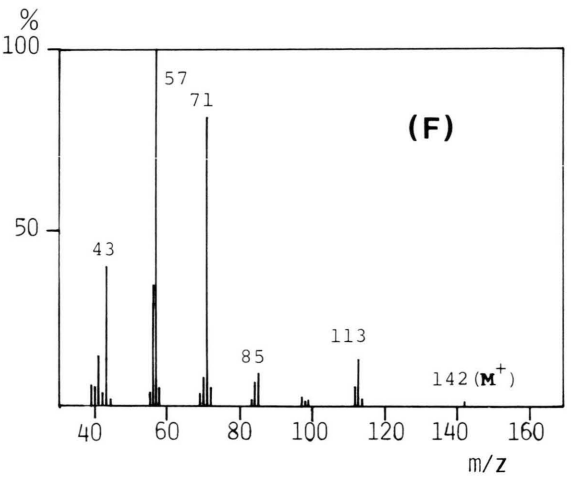


Fig. 3. Mass spectra of hexahydroocimene (E) and hydrogenation product of the scent substance of male wings of *Hebomoia glaucippe liukiuensis* (F).

Table I. Constituents of the essential oil of *Crataeva religiosa*.

Peak No.	Components	Mass spectra						Yield [%]
		M^+	H_1	H_2	H_3	H_4	H_5	
1	2-hexanal	98	83	69	98	70	80	50.3
2	3-hexen-1-ol	100	67	82	69	70	83	40.2
3	*p*-cymene	134	119	134	120	91	135	0.5
4	limonene	136	68	93	136	67	121	0.6
5	linalool	154	93	80	68	71	121	0.6
6	α-ionone	192	121	136	93	192	109	0.2
7	β-ionone	192	177	178	192	149	159	0.2

M^+; molecular ion, H_1; base peak, H_2; second-highest ion, H_3; 3rd-highest ion, H_4; 4th-highest ion, H_5; 5th-highest ion.

Table II. EAG responses of adult male and female antennae of *Hebomoia glaucippe liukiuensis.*

Stimuli	Response [mV]	
	Male	Female
Limonene	0.14	0.10
Myrcene	0.11	0.15
E-β-Ocimene	0.38	0.16
Z-β-Ocimene	0.31	0.15

The contents of the ocimene in the wings and bodies of both sexes of *H. glaucippe liukiuensis* are as follows: male wings, 1.8 μg per one head; female wings, 0.04 μg; male body, 167.6 μg; and female body, 155.7 μg. Those of *H. glaucippe formosana* are similar to above. The content of *E*-β-ocimene was more than 95% of the total extracts. The quantity of the ocimene was largest at the body and a trace amount of the ocimene was detected from the wings of adult male and female.

Identification of the volatile constituents of the food plants, Crataeva religiosa

The analytical results of the essential oils of the food plants by means of GC and GC-MS are shown in Table I. The main volatile constituents of the leaves are found to be 2-hexanal and 3-hexen-1-ol together with a small amount of *p*-cymene, limonene, linalool, and α,β-ionones [5].

EAG measurements

EAG were measured both sexes to ocimene, limonene, and myrcene (Table II). EAG responses of male butterflies to ocimene were greater than those of limonene and myrcene, and male antenna responded strongly to ocimene rather than females.

Acknowledgements

Authors are indebted to Mr. Yoichi Tanaka for his help in collecting butterflies (in part) and plant material.

[1] G. Bergstrom and L. Lundgren, Zoon Supply **1,** 67 (1973).
[2] N. Hayashi, Y. Kuwahara, and H. Komae, Experimentia **34,** 684 (1978).
[3] Y. Kuwahara, Appl. Ent. Zool. **14,** 350 (1979).
[4] G. Ohloff, J. Seibl, and E. sz. Kovats, Liebigs Ann. Chem. **675,** 83 (1964).
[5] Spectral Atlas of Terpenes and the Related compounds, ed. by Y. Yukawa and S. Itō, Hirokawa Publishing Co., Tokyo 1973.