Analyses of lepidopteran sex pheromones by mass spectrometry

Tetsu Ando, Rei Yamakawa

Lepidoptera, including about 150,000 species in the world, comprise the second largest insect group, and sex pheromones have been identified from virgin female moths of more than 600 species. The chemical structures are simple, but diverse, because species-specific pheromones play an important role in the reproductive isolation of each species. The pheromone content in each female is quite low, and gas chromatography coupled to mass spectrometry (GC-MS) is most frequently utilized to reveal the chemical structure. Almost all pheromone components are straight-chain compounds and are classified into two major groups [i.e. unsaturated C_{10}–C_{18} fatty alcohols and their derivatives (Type I) and C_{17}–C_{23} polyenyl hydrocarbons and their epoxides (Type II)]. In addition to the unbranched compounds, some species secrete methyl-branched compounds (e.g., 2-ketones). For the identification of these compounds, determining the positions of the double bond, the epoxy ring, and the methyl group is an important key step. Copious spectral information measured by electron-impact ionization (70 eV) has been accumulated for these compounds. This review therefore deals with their spectral characteristics, namely, diagnostic ions, to apply them to pheromone studies on new target insects.

Keywords: Diagnostic fragment ion; Epoxide; Fatty alcohol; Female moth; GC-EI-MS; Lepidoptera; Methyl-branched ketone; Polyene; Sex pheromone; Structure determination

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1. Diversity of lepidopteran sex pheromones

Lepidoptera comprise the second largest insect group, which includes about 150,000 species in the world. The sex pheromone, which is secreted by the female moth as a chemical cue in mating communication, has strong activity to attract the male moth of the same species. This species-specific pheromone prevents inter-species crossing and plays an important role in reproductive isolation. The first pheromone study was accomplished with a very large number of female moths of the silkworm in the late 1950s [1]. Since the identification of bombykol, female sex pheromones have been reported for more than 600 moth species, including many agricultural pest insects [2,3]. In order to develop a system for integrated pest management, synthetic pheromones are utilized not only as a monitoring tool in place of a luring lamp but also for mass trapping and disruption of mating communication in the field [4].

Fig. 1 shows the chemical structures of some representative lepidopteran pheromones. Primary alcohols and their derivatives (mainly acetates and aldehydes) with a long straight chain (C_{10}–C_{18}) have most commonly been detected in pheromone-gland extracts of lepidopteran females. The compounds in this most dominant group, Type I pheromones, comprise about 75% of the known pheromones and have been detected widely from species in all of the main taxonomical groups of moths {e.g., the silk moth (family: Bombycidae), the smaller tea tortix (Tortricidae) [5], and the citrus leafminer moth (Gracillariidae) [6–9]}. Polyunsaturated hydrocarbons and their epoxy derivatives with a longer straight chain (C_{17}–C_{23} and, exceptionally, C_{25} and C_{27}) comprise a second major group, Type II pheromones. The pheromone components, lacking a functional group at the terminal position, represent about 15% of the known lepidopteran pheromones, and have been identified from insects in highly evolved groups {e.g., the giant looper (Geometridae) [10], the tussock moth (Limantriidae) [11], and the fall webworm (Arctiidae) [12]}. In addition to Type I and II pheromones, secondary alcohols and ketones with a straight chain and esters of a long unbranched-chain acid have been

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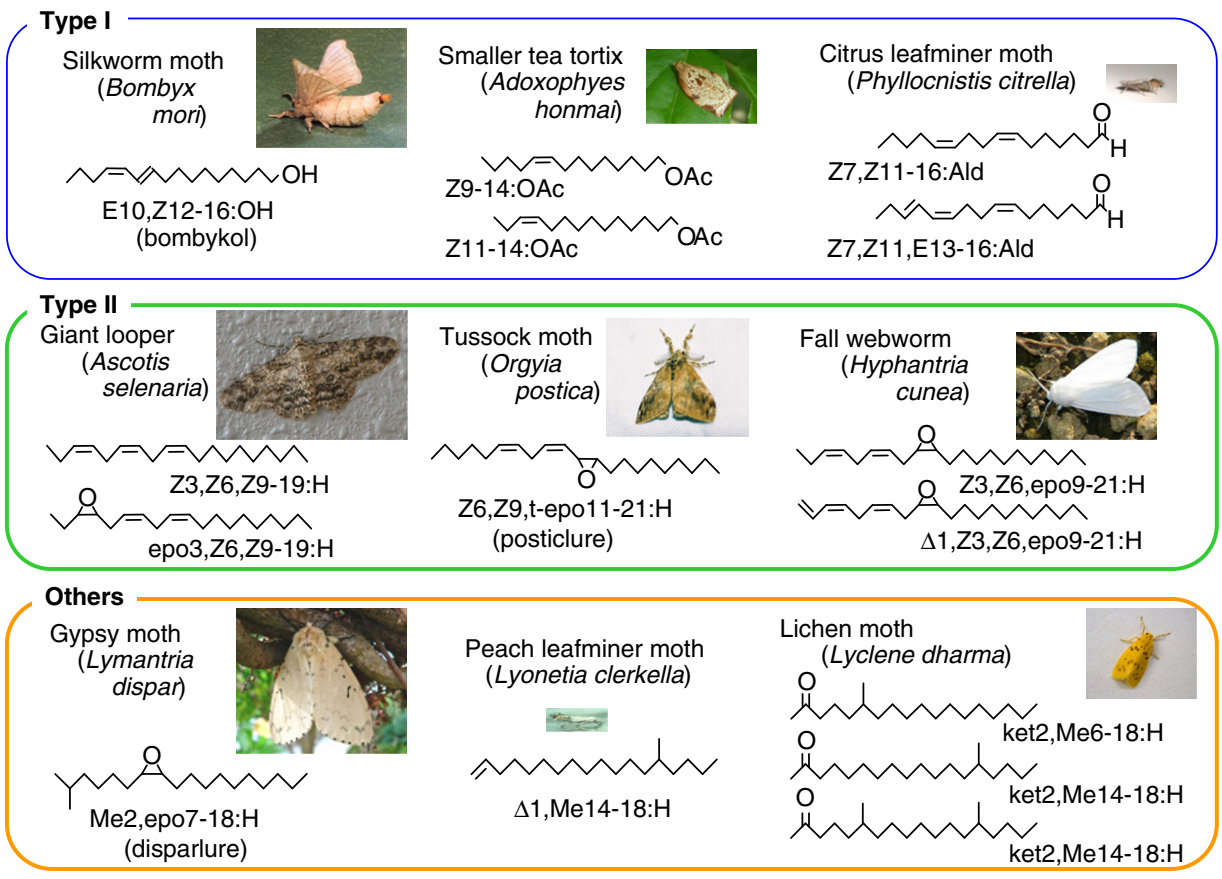

Figure 1. Representative lepidopteran sex pheromones. Compounds are abbreviated as follows: Z = (*Z*)-double bond; E = (*E*)-double bond; Δ = terminal double bond; the number before the hyphen = position of the double bond, epoxy ring, or methyl branch; number after the hyphen = the carbon number of the straight chain; OAc = acetate; OH = alcohol; Ald = aldehyde; H = absence of a terminal functional group; epo = *cis*-epoxy ring; *t*-epo = *trans*-epoxy ring; Me = methyl branch; and, ket = keto group. Pictures of insects were kindly supplied by Utsugi Jinbo and Takayuki Suzuki (http://www.jpmoth.org/).

identified from several species [4]. Furthermore, some species produce methyl-branched compounds {e.g., an epoxide of the gypsy moth (Limantriidae) [13], a hydrocarbon of the peach leafminer moth (Lyonetiidae) [14], and 2-ketones of the lichen moth (Arctiidae) [15,16]}.

It is likely that each species designs the individual structure of its own pheromone differently by changing the carbon number of the chain, the kind of the functional group, and/or the number, the position, and the configuration of a double bond and a methyl group. However, variation of the chemical structure is limited. The diversity of the lepidopteran sex pheromones is generated by blending several components [4]. Innumerable pheromone blends are based on combinations of different components and on variations in the mixing ratio. Usually, a minor component is indispensable to attract males. In addition to the structures of every pheromone component, an instrumental analysis of the pheromone should clarify the exact mixing ratio produced by the female.

2. General procedure for pheromone identification

The activity of the sex pheromone is measured by an electrophysiological technique as well as by male responses observed in a flask or a wind tunnel. The electroantennogram (EAG), a recording of potential changes measured between the base and the tip of an antenna as a result of chemical stimulation [17], can be utilized as a bioassay system. Since the male antenna of each species specifically and supersensitively responds to the pheromone secreted by the corresponding female, species-specific perception can be recorded. One of the advantages of the system is that the EAG is recorded against each compound of a multi-component pheromone even where male attraction is induced by a pheromone blend [18]. The activities of several compounds can be measured after short intervals by using one unconditioned antenna in a well-lit room. All active compounds eluted from a capillary GC column are successively detected with an instrument using the EAG technique as a detector (EAD) [19], so many pheromone