Osmeterial Secretions of Larvae of Papilionid Butterflies (Lepidoptera: Papilionidae)

I. Identification of Myrcene from *Luehdorfia japonica* and *L. puziloi inexpecta*

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Myrcene was identified by TLC, GLC and GC-MS from the osmeterial secretions of the larvae of the papilionid butterflies, *Luehdorfia japonica* and *L. puziloi inexpecta* (subfamily Parnassinae, tribe Zerynthiini). This is the first report of the presence of mono-terpene in osmeterial secretions of papilionid caterpillars, and also is the first identification of a principal component of the secretions from the members of subfamily, Parnassinae.

INTRODUCTION

Luehdorfia japonica LEECH and the closely related species, *L. puziloi inexpecta* SHELJUZHKO are very beautiful butterflies found in early spring. Both species belong to the members of subfamily Parnassinae, tribe Zerynthiini. The former lives only in Japan. The latter is subspecies distributed in the main island of Japan.

Papilionid caterpillars secrete defensive liquids from their osmeteria when they are stimulated. Isobutylic acid and 2-methylbutyric acid have been identified from osmeterial secretions of many species of papilionid caterpillars (EISNER and MEINWALD, 1965; EISNER et al., 1970; LOPEZ and QUESNEL, 1970; OSHIMA et al., 1975). However, no other compounds have been known except these acids until today. OSHIMA et al. (1975) studied on the secretions of eight species of Japanese papilionid caterpillars including *Luehdorfia*, and reported that these acids were not present in both species of *Luehdorfia*.

We have identified myrcene from both *Luehdorfia* species, and report the result in this paper.

MATERIALS AND METHODS

Insects. The *Luehdorfia* butterflies were collected in the field. Their eggs were obtained by the hand-pairing technique. The larvae were reared on their food-plants, *Heterotropa tamaense* (for *L. japonica*) and *Asiasarum sieboldii* (for *L. puziloi inexpecta*).

Collection of the osmeterial secretions. A small glass capillary was inserted into the osmeteria of the fifth-instar larvae of each species, and the secretions were soaked up. The secretions (total eight larvae of each species) were dissolved in hexane (50 μ l).

Hexane-insoluble materials were present, but the characteristic odor of the secretions was found in hexane-soluble fraction.

Thin-layer chromatography (TLC). TLC was carried out under following condition: Merk Silica-gel HF₂₅₄, 0.5 mm thickness, hexane-ether (9:1, v/v) as developing solvent.

Gas liquid chromatography (GLC). A Shimadzu GC-6A gas chromatograph equipped with a hydrogen flame ionization detector using 3 mm × 2 m column packed with 5% SE-30 or 5% DEGS, both on Chromosorb W (AW, 80–100 mesh) was used. The column temperature was 70°C (SE-30) or 60°C (DEGS). n-Decane was used as standard material.

Mass spectrometry (MS). Mass spectra were measured with a Hitachi RMU-6M GC-mass spectrometer equipped with a 3 mm × 2 m glass column packed with 2% OV-1 on Chromosorb W at 20 eV (60°C).

Catalytic hydrogenation of the hexane extract. A part of the hexane extract of the secretions was hydrogenated under hydrogen atmosphere by use of PtO₂ catalyst.

RESULTS AND DISCUSSION

A large spot (Rf 0.73) and very small spot (original point) were observed in the thin-layer chromatoplate. Under the same condition, trans-stilbene (standard) was detected at Rf. 0.64. Therefore, the large spot was considered to be hydrocarbon. The gas liquid chromatograms of the hexane extracts revealed the presence of at least five components as shown in Fig. 1. The content of the major component was more than 95% in both species. The retention times (Rt) of the main peak material were 7.36 min (SE-30; under the same condition, n-decane 8.38 min) and 5.40 min (DEGS; n-decane, 3.80 min), respectively. The mass spectrum of the major component was shown in Fig. 2. The molecular ion peak was observed at 136, and the spectrum agreed with that of myrcene (C₁₀H₁₆) described in the literature (ENZELL et al., 1972). To confirm it, the mass spectrum of authentic myrcene was measured under the same condition. Both spectra were identical. The mass spectrum of the hydrogenation product of the major component of the secretion (Fig. 3) was also identical with that of the product obtained from authentic myrcene. In addition, the characteristic odor of the secretions was similar to that of myrcene. From these data, the major component of the osmeterial secretions of both *Luehdorfia* was concluded to be myrcene.

This is the first report of the presence of mono-terpene in osmeterial secretions of papilionid caterpillars, and also is the first identification of a principal component of the secretions from the members of subfamily, Parnassinae. EISNER et al. (1970) indicated that the larvae of *Battus polydamas* (L.) (subfamily Papilioninae, tribe Troi-dini) secreted a mixture containing two

Fig. 1. Gas chromatograms of the hexane extracts of the osmeterial secretions of *Luehdorfia japonica* (I) and *L. puziloi inexpecta* (II). Condition: 5% SE-30 on Chromosorb W (AW, 80–100 mesh, 2m × 3mm), col. temp., 70°C.

Fig. 2. Mass spectrum of the major component of the secretion of *L. japonica* (myrcene)

Fig. 3. Mass spectrum of the hydrogenation product of the major component (hexahydro-myrcene)

unidentified sesquiterpenes instead of two acids mentioned above.

Myrcene occurs in many plants, and it is not clear that whether myrcene in the secretions is biosynthesized by the larvae themselves or derived from their food-plants.

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