effective antagonist of PMA-induced mouse ear inflammation (Dose PMA = 2 µg/ear): topical application of luffolide at 50 µg/ear significantly inhibited inflammation (55% inhibition). The hydrolysis of phosphatidylcholine by bee venom PLA_2 is completely inhibited by luffolide at a concentration of 3.5 µM (100%). These data may shed some light on the chemical structure of the covalent adduct between manoalide (**1**) and PLA_2. It has been proposed[7] that a lysine residue of PLA_2 reacts at C-6 of manoalide in a Michael addition to the α, β-unsaturated aldehyde that results from opening the pyran ring. The fact that luffolide (**4**) has a saturated aldehyde in place of the 'unsaturated aldehyde' of manoalide suggests that the lysine residue may react at the aldehyde group to form, at least initially, a Schiff base[8].

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2 On leave from Central Chemical Research Organization, Budapest, Hungary.

3 de Silva, E. D., and Scheuer, P. J., Tetrahedron Lett. *21* (1980) 1611; de Silva, E. D., and Scheuer, P. J., Tetrahedron Lett. *22* (1981) 3147.
4 Jacobs, R. S., Culver, P., Langdon, R., O'Brien, T., and White, S., Tetrahedron Lett. *41* (1985) 981; Glaser, K. B., and Jacobs, R. S., Biochem. Pharm. *35* (1986) 449; Glaser, K. B., and Jacobs, R. S., Biochem. Pharm. *36* (1987) 2079; Lombardo, D., and Dennis, E. A., J. biol. Chem. *260* (1985) 7234; Deems, R. A., Lombardo, D., Morgan, B. P., Mihelich, E. D., and Dennis, E. A., Biochim. biophys. Acta *917* (1987) 258.
5 Albizati, K. F., Holman, T., Faulkner, D. J., Glaser, K. B., and Jacobs, R. S., Experientia *43* (1987) 949.
6 All crystallographic calculations were done on a PRIME 9950 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: FOBS a data reduction program by G. D. Van Duyne, Cornell University, 1987; MULTAN 80, and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq and M. M. Woolfson, University of York, England, 1980; BDLS, an anisotropic block diagonal least squares refinement written by K. Hirotsu, E. Arnold, and G. D. Van Duyne, Cornell University, 1987; PLUTO 78, a locally modified crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu and G. Van Duyne, Cornell University, 1985.
7 Reynolds, L. J., Morgan, B. P., Hite, G. A., Mihelich, E. D., and Dennis, E. A., J. Am. Chem. Soc. *110* (1988) 5172.
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Protective chemicals in caterpillar survival

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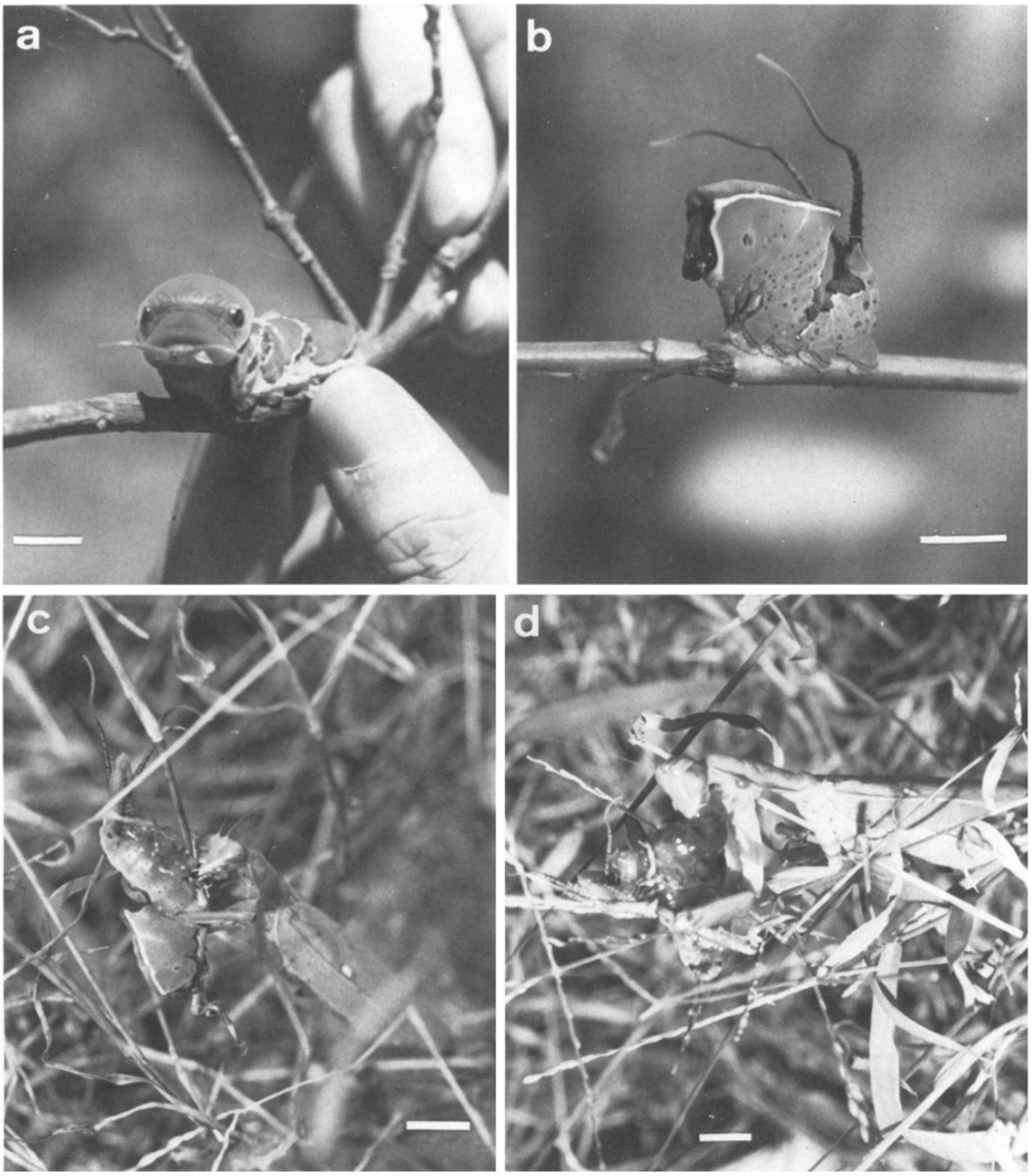
Summary. Using two species of caterpillars; larvae of the swallowtail butterfly, which produce large amounts of iso-butyric and 2-methylbutyric acids, and larvae of the puss moth, which produce no such detectable volatile compounds, it was shown that the insect which utilizes a chemical defense is better protected from its natural enemy, a praying mantis.
Key words. Iso-butyric and 2-methylbutyric acids; *Papilio memnon heronus; Cerura erminea menciana; Hirodula patellifera;* osmeteria; mimicry.

In Taiwan, during the summer, we can find two types of lepidopterous larvae with eversible tissues used in defense behavior. One, the caterpillar of the swallowtail butterfly *Papilio memnon heronus* Frunstorfer, has an eversible cervical gland or osmeterium in an anterior position on the head, whereas the larva of the puss moth, *Cerura erminea menciana* Moore, has an eversible tube in a posterior position on the abdominal end (fig., a and b). Although in response to irritation the hidden structures of both these species will evert, and the same bright red color becomes visible, only the former species seemed capable of producing a strong odor. Therefore, we hypothesized that the larva of the puss moth may mimic the caterpillar of the swallowtail butterfly, to avoid their common predator, the praying mantis *Hirodula patellifera* Serville. Close mimicry, where a harmless species mimics the warning coloration of one that harms or sickens predators, is very common in caterpillars[1, 2], so these 2 insects are good subjects for experiment. The results we report here, however, show that in the case of the puss moth, caterpillar mimicry is not a valuable protection against one of its natural predators, the praying mantis.

Caterpillars of *Papilio memnon heronus* were collected from the Yangmingsan area in the vicinity of Taipei. Secretions discharged from the osmeterial glands of 25 individuals were dissolved in methylene chloride. One µl of the extract, corresponding to one tenth of an individual discharge, was injected into a 3% OV 101 capillary



a Larvae of *Papilio memnon heronus* fully everting its osmeterium in response to a sudden disturbance. *b* Larva of *Cerura erminea menciana* Moore showing its two horns with soft tissue (tube) extruded from the rear end. *c, d* Sequential pictures of a *Cerura* larva being consumed by a praying mantis *Hirodula patellifera* in the presence of the soft tissue (arrow). Reference bars: 1.0 cm.

column in a Varian Aerograph Series 3700 gas chromatograph, under conditions similar to those described in a previous report[3]. In addition to 3 small peaks, 2 large asymmetrical peaks were obtained. The compounds in these 2 large peaks had an odor which we perceived as sour or pungent. In the gas chromatographical analysis they showed high polarity in the non-polar column. The secretion was injected again into a polar column (GP 60/80 Carbopack C/0.3% Carbowax, 20M/0.1% H_3PO_4) for organic acid determination. The retention times of the 2 compounds were identical with those of authentic iso-butyric acid and 2-methylbutyric acid.

Another 1 µl of the secretion was injected into a SE-54 column (5% phenyl film thickness 0.25 µm 30 m) connected with an ion trap detector (Finnigan 700). The first peak gave major fractions at m/z 43 (100%), 49, 55, 60, 73, 84 and the quasimolecular ion (QM^+) 89, and the second peak at m/z 39, 41, 45, 49, 53, 57, 69, 74 (100%), 85, 87, 89, 93 and the quasimolecular ion 103. All spectral data coincided with those of the acids mentioned above. Iso-butyric acid and 2-methylbutyric acid were first reported from the American swallowtail butterfly *Papilio machaon*[4, 5], and their corresponding methyl esters from the African swallowtail *P. demodocus*[6]. The chemical components of the osmeterial secretions of six *Papilio* species at different stages have also been documented, and the major components iso-butyric acid and 2-methylbutyric acid in the 5th (last) instar larvae were found to vary very little in quantity among them[7, 8]. Our result using a Chinese species confirms this previous identification. The procedures of isolation and identification of the volatile acids were repeated again with the eversible tube of the puss moth caterpillar, but no peaks were obtained.

The biological function of iso-butyric acid and 2-methylbutyric acid as a defensive secretion was first demonstrated against ants[4], and recently against other small natural enemies, such as salticid spiders[9]. In Taiwan the large predator, the praying mantis *Hirodula patellifera* Serville, is usually found in citrus orchards and willow trees. Female mantids were considered the most suitable subjects for the experiments on predation; a female mantis may even bite the head off her unfortunate mate in some sexual encounters[10]. Ten caterpillars of the above mentioned species were exposed to 5 female praying mantids for 3, 6, 9, 12 and 24 h in natural habitats. The results are presented in table 1. From these data it can be seen that only the *Papilio* larvae (90%) with the acid secretions could escape from the attack of hungry mantids. All the *Cerura* larvae (100%) were dead within 24 h. Because the difference of the result is so evident, no statistical analysis is needed. Figure c and d clearly demonstrates the fact that the mantis is eating the larva despite the presence of the extruded tubes.

Therefore the tube had no defensive value against mantis. It may, however, have a function in defense against other vertebrate predators (birds) or *Cotesia* parasitoids[11]. It has been recorded that many notodontid species emit formic acid from cervical glands[12]. We examined our *Cerura* larvae, and observed a small prothoracic gland at the cervical margin of the prosternite. The quantity of formic acid secreted, as determined by our method, was less than one-tenth of the isobutyric acid secreted by *Papilio* larvae. So the formic acid secreted from *Cerura* larvae is probably not enough to defend them against the mantis. In order to clarify this further, 2 *Papilio* larvae and 2 *Cerura* larvae of the same size were released into a plastic box (25 cm × 25 cm × 25 cm) with host plant leaves as food. Then a praying mantis was introduced. One week later only *Papilio* larvae survived. In a survey carried out in 1987 throughout Taiwan island, we found that the natural population of *Papilio* caterpillars is higher than that of *Cerura* caterpillars (table 2). These results might suggest that simple fatty acids, such as isobutyric and 2-methylbutyric acids, are good defensive chemicals. However, the mimicry of the *Papilio* caterpillar by that of *Cerura* does not appear to be an effective mechanism for protecting the latter against predation by mantids, although such mechanisms have been shown to function in other species.

Table 1. Mortality of the ten 5th-instar larvae released after 5 female praying mantids' attack

	Individual mortality (%) hours after release 3	6	9	12	24
Papilio memnon	10	10	10	10	10
Cerura erminea	10	20	40	60	100

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1 Wickler, W., Mimicry in Animal and Plants. Trans. from the German by R. D. Martin. World University Library, Weidenfield and Nicolson, London 1968.
2 Starr, C., and Taggart, R., Biology, the Unity and Diversity of Life, pp. 749–761. Wadsworth Pub. Company, Belmont, California 1986.
3 Chow, Y. S., and Lin, Y. M., J. ent. Sci. *21* (1986) 97.
4 Eisner, T., and Meinwald, Y. C., Science *150* (1965) 1723.
5 Eisner, T., Pliske, T. E., Ikeda, M., Owen, D. F., Vazguez, L., Perez, H., Franclemont, J. G., and Meinwald, J., Ann. ent. Soc. Am. *63* (1970) 914.
6 Burger, B. V., Roux, M. L., Spies, H. S. C., Truter, V., and Geertsema, H., J. Insect Physiol. *24* (1978) 803.
7 Honda, K., J. Insect Physiol. *26* (1980) 39.
8 Honda, K., J. chem. Ecol. *6* (1981) 1089.
9 Damman, H., Ecol. Ent. *11* (1986) 261.
10 Kyriacou, C. P., Trends Ecol. Evol. *2* (1987) 350.
11 White, T. R., Weaver, J. S. III, and Agee, H. R., Ann. ent. Soc. Am. *76* (1983) 1.
12 Weatherston, J., Percy, J. E., MacDonald, L. M., and MacDonald, J. A., J. chem. Ecol. *5* (1979) 165.

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Table 2. The time of presence of the larvae on ten host plants examined at different locations of Taiwan in 1987

	Yangmingsan Mar. 29	Jun. 7	Sept. 13	Nov. 22	Nankang Mar. 28	Jun. 6	Sept. 12	Nov. 21	Taichung Sept. 20	Nov. 29
*Papilio memnon**	0	3	7	5	0	2	4	10	2	3
Cerura erminea #	0	0	3	0	0	0	8	10	0	0

* Presence of larvae per 10 citrus trees. # Presence of larvae per 10 willow trees.