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A CHEMICAL BASIS FOR DIFFERENTIAL ACCEPTANCE OF *Erysimum cheiranthoides* BY TWO *Pieris* SPECIES

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Abstract—Wormseed mustard, *Erysimum cheiranthoides*, is unacceptable as a host for the cabbage butterfly, *Pieris rapae*. However, it is preferred for oviposition by *Pieris napi oleracea* in the greenhouse. Isolation and identification of the oviposition stimulants to *P. napi oleracea* were accomplished by C_{18} open-column chromatography, TLC, ion-exchange chromatography, HPLC, UV, and NMR spectroscopy. Glucoiberin and glucocheirolin were identified as the most active stimulants. The extracted glucoiberin was as stimulatory as glucocheirolin, although its concentration in the *Erysimum* plants was about 10 times lower than that of glucocheirolin. These glucosinolates were only weak stimulants to *P. rapae*. Furthermore, *P. rapae* was strongly deterred by the cardenolides, erysimoside and erychroside, from *E. cheiranthoides*, and *P. napi oleracea* was less sensitive to these compounds. No other deterrent to *P. napi oleracea* was detected in this plant species. The results explain the differential acceptance of *E. cheiranthoides* by these two *Pieris* species.

Key Words—*Pieris rapae*, *Pieris napi oleracea*, Lepidoptera, Pieridae, *Erysimum cheiranthoides*, oviposition, stimulants, deterrents, glucosinolates, glucoiberin, glucocheirolin, cardenolides.

INTRODUCTION

The cabbage butterflies, *Pieris rapae* L. and *P. napi oleracea* Harris, have overlapping but distinct host ranges (Richards, 1940; Chew, 1977a,b). Previous comparative studies using 11 potential host-plant species under controlled con-

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ditions (Huang and Renwick, 1993) showed that some plant species such as cabbage were acceptable as host plants for *Pieris* species, but that preferences for other plants were quite different. Wormseed mustard, *Erysimum cheiranthoides*, was unacceptable to *P. rapae*, but was strongly preferred by ovipositing *P. napi oleracea*. In a 10-day no-choice oviposition bioassay, *P. rapae* refused to oviposit on *E. cheiranthoides* plants throughout their life, whereas *P. napi oleracea* laid a large number of eggs. *P. napi oleracea* laid significantly more eggs on *E. cheiranthoides* than on cabbage plants in two-choice bioassays (Huang and Renwick, 1993). These observations suggest that the oviposition stimulants to *P. napi oleracea* in *E. cheiranthoides* are much more potent than those in cabbage and that the acceptance or rejection of *E. cheiranthoides* by the two *Pieris* species is probably influenced by different classes of chemicals. However, we still do not know to what extent plant chemistry controls host specificity (Bernays and Graham, 1988; Thompson, 1988). Investigation into the chemical basis of differential selection of host plants by the two *Pieris* species may provide useful information on the mechanism of host specificity of herbivorous insects.

Studies have been made to document the chemical factors regulating the avoidance of *E. cheiranthoides* by *P. rapae*. Renwick and Radke (1985,1987) found that a polar extract of *E. cheiranthoides* contained oviposition stimulant(s) as well as deterrents to *P. rapae*. The deterrents were subsequently identified as the cardenolides, erysimoside and erychroside (Renwick et al., 1989; Sachdev-Gupta et al., 1990), but the stimulants have yet to be identified. Other plant secondary compounds such as coumarin and rutin also deter oviposition by *P. rapae* (Tabashnik, 1987). No study has been conducted to determine how *P. napi oleracea* responds to the cardenolides or if there are other deterrents in the plants. Furthermore, the oviposition stimulants to *P. napi oleracea* from *E. cheiranthoides* are unknown.

The study reported here was designed to determine whether known cardenolides from *E. cheiranthoides* act as oviposition deterrents to *P. napi oleracea*, to identify the oviposition stimulants from the same plant species, to measure the relative sensitivities of *P. rapae* and *P. napi oleracea* to the deterrents and the stimulants, and finally to explain chemically the differential acceptance of the plants by the two *Pieris* species.

METHODS AND MATERIALS

Insects and Plants. P. rapae and *P. napi oleracea* butterflies for behavioral assays were obtained from colonies started from field-collected insects each summer and maintained in the laboratory at ca. 22°C under fluorescent lights providing a photoperiod of 16:8 hr light–dark. Oviposition occurred in the greenhouse, with supplementary lighting, at ca. 25°C. *P. rapae* larvae were

reared on cabbage (*Brassica oleracea* L. var. Golden Acre) and *P. napi oleracea* on *Conringia orientalis* plants. Pupae were separated by sex (Richards, 1940) and kept in screen cylinders until eclosion. *E. cheiranthoides* and cabbage plants (4–6 weeks old) for extraction were grown in an air-conditioned greenhouse at ca. 25°C. Supplemental light was provided by 400-W multivapor high-intensity discharge lamps.

Extraction of Plant Materials. Fresh foliage was extracted in boiling ethanol for 5 min, cooled, homogenized, and filtered. The ethanolic extract was evaporated to dryness under reduced pressure and then defatted with *n*-hexane. The defatted extract was dissolved in water and extracted three times with *n*-butanol. The butanol extract and the postbutanol water extract were concentrated under reduced pressure at ca. 50°C and kept in the refrigerator.

Isolation of Active Compounds. The postbutanol water extract was found to contain strong oviposition stimulant(s) to *P. napi oleracea*. The active material was therefore separated by atmospheric-pressure chromatography using a 45 × 2-cm reversed-phase column packed with 30 g 55 to 105-μm preparative C_{18} (Millipore Corporation, Milford, Massachusetts). After loading the sample (200 gram leaf equivalents), the column was sequentially eluted with 0.5% potassium sulfate (150 ml), water (150 ml), 25% (15 ml), 33% (15 ml), 50% (15 ml), and 100% (45 ml) methanol in water, and 26 fractions (15 ml each) were collected. Different combinations of these fractions were tested for stimulatory activity. Preparative thin-layer chromatography (TLC) of active fractions (fractions 6–8) was carried out on 20 × 20-cm, 0.5-mm-thick, Merck silica gel 60 plates. Twenty gram leaf equivalents of the sample was loaded on each plate in a line, and the plate was developed in a solvent system consisting of ethyl acetate–methanol–acetic acid–water (4 : 1 : 1 : 0.5). The plates were dried with a hair-drier immediately after development. A small portion of the plate was cut off and bands were visualized by spraying with 1% ceric sulfate solution followed by heating at 110°C for ca. 15 min. Corresponding bands on the untreated portion were individually collected and washed with methanol. The eluted material was used for oviposition bioassays or glucosinolate analyses.

Cardenolides (erysimoside, erychroside, and erycordin) were isolated as a combination from the butanol extract by HPLC as previously described (Renwick et al., 1989; Sachdev-Gupta et al., 1990) using a water–acetonitrile gradient. A semipreparative reversed-phase C_{18} column (50 cm × 8 mm) was used and the flow rate was maintained at 3.3 ml/min. The solvent composition increased from the initial pure water to 23% CH_3CN at 10 min, 35% CH_3CN at 40 min and 100% CH_3CN at 45 min. The eluate was monitored at 219 nm.

Bioassays. Oviposition bioassays were conducted in screen cages (48 × 48 × 48 cm) in a greenhouse as described by Renwick and Radke (1988). Eight pairs of newly emerged butterflies were transferred to each cage in the greenhouse. Each cage was supplied with a vial of 10% sucrose solution containing

yellow food coloring and a cotton wick to facilitate feeding. During the preoviposition period, a cabbage plant was placed in each cage. When more than 50 eggs were observed, the plant was removed and the butterflies were used for testing the next day. Treated and control plants were placed in opposite corners of the cage. Positions of plants were alternated in each cage to control for possible position effects. However, the plants were randomly arranged in a circle when three or more test plants were included in each cage, as when the activities of fractions from a column were compared. Bioassays were started at 9:30 a.m. and the eggs laid were counted at 3:30 p.m. When a comparison between the two *Pieris* species was necessary, both species were tested at the same time to minimize the possible effects of differences between plant batches, intensity of sunlight, and other factors on oviposition behavior. Treated plants were sprayed with samples dissolved in a methanol–water combination (70 or 80% methanol depending on solubility of the samples). Control plants were sprayed with cabbage postbutanol water extract (as a standard of stimulant) or solvent alone. The solutions were applied in a fine mist with chromatographic sprayer to both upper and lower leaf surfaces. The concentrations applied were expressed as gram leaf equivalents of the original plant foliage. Deterrent activity was monitored by applying test solution on cabbage plants grown individually in 10-cm cord pots. In separate bioassays, stimulatory effects were tested using neutral (stimulants were not present) bean (*Phaseolis vulgaris* var. Sieva) plants as the oviposition substrate. The bean plants used for bioassays were grown under the same conditions as for cabbage plants and presented as single plants at the two-leaf stage (ca. 20 days old) in plastic pots (6.25 × 6.25 cm).

Design and Analysis. A randomized complete block design was used in the bioassays. A replication consisted of one cage with eight pairs of butterflies, and four to nine replications were performed for each bioassay. When cabbage postbutanol water extracts were used as controls and when comparisons between bioassays were necessary, the relative stimulatory activities were presented as an oviposition stimulant index (OSI), where OSI = 100 (treated − control)/(treated + control).

Deterrent activities were compared by calculating an oviposition deterrent index (ODI), i.e., ODI = 100 (control − treated)/(control + treated).

A paired t test or a Waller-Duncan K-ratio t test was used to assess significance of differences between treatments and controls or among treatments.

HPLC of Desulfoglucosinolates. Desulfated samples were prepared to test for the presence of glucosinolates, according to the method of Minchinton et al. (1982). A column was packed in a 12.7-cm Pasteur pipet with 200 mg DEAE Sephadex A-25 in 0.5 mol pyridine–acetate buffer. The column was conditioned with the buffer (6 ml) followed by water (6 ml). After loading the samples (5–10 gram leaf equivalents in 0.5–1.0 ml water), the column was eluted with water (10 ml or until the eluate was colorless), and 1 ml 0.25% aqueous solution

of sulfatase (Sigma Chemical Co.) was applied. The column was kept at room temperature overnight and then eluted with 2 ml water. The desulfated products were filtered and analyzed by HPLC on a reversed-phase C_{18} column (25 × 0.46 cm) using a gradient program as follows: 0% CH_3CN in water at 0 min, 10% at 35 min, 20% at 60 min, and 100% at 70 min. The flow rate was maintained at 1 ml/min. A diode array detector (Hewlett Packard model 1040A) was used to monitor the eluate at 219 nm.

Identification. UV spectra of isolated compounds were obtained from the diode array detector in water-acetonitrile.

Based on the TLC, HPLC, and UV spectra of the desulfated samples, the two stimulants were found to be aliphatic glucosinolates with similar polarities. To readily obtain a sufficient sample of these stimulants for NMR spectroscopy, desulfation of the postbutanol water extract was performed. The desulfation procedure was similar to that described above. However, larger amounts of DEAE Sephadex A-25 (4 g), plant extract (200 gram leaf equivalents), and sulfatase (20 ml 0.25% aqueous solution) were used. The desulfated glucosinolates were individually collected by HPLC on a semipreparative reversed-phase C_{18} column (50 cm × 8 mm) using a linear water–acetonitrile gradient from pure water to 15% CH_3CN in 30 min at a flow rate of 3.3 ml/min. Solvent was evaporated from collected material under reduced pressure at ca. 55°C, and the desulfoglucosinolates were analyzed by ^{13}C NMR spectroscopy. The samples were dissolved in methanol-d_4–pyridine-d_5 (9:1). The spectra were recorded at ambient temperature on a Varian XL-400 instrument. The chemical shifts were indirectly referenced to tetramethylsilane by using methanol-d_4 as an internal reference.

RESULTS

P. napi oleracea laid significantly more eggs on bean plants treated with the *E. cheiranthoides* postbutanol water extract than on control bean plants treated with solvent (70% MeOH) alone (Figure 1). The number of eggs laid by *P. rapae* on the test plants was not significantly different from that on control plants. This indicates the presence of strong oviposition stimulant(s) for *P. napi oleracea* in the extract and a relative insensitivity of *P. rapae* to the extract components. Therefore, subsequent bioassays of fractions and purified compounds were carried out using only *P. napi oleracea*.

Oviposition bioassays of five combinations of fractions from the atmospheric-pressure column revealed that fractions 6–10 were most stimulatory to *P. napi oleracea* (Figure 2). Fractions 1–5 were also stimulatory, but significantly fewer eggs were laid when compared with fraction 6–10. All the other combinations were not significantly stimulatory to *P. napi oleracea* when com-

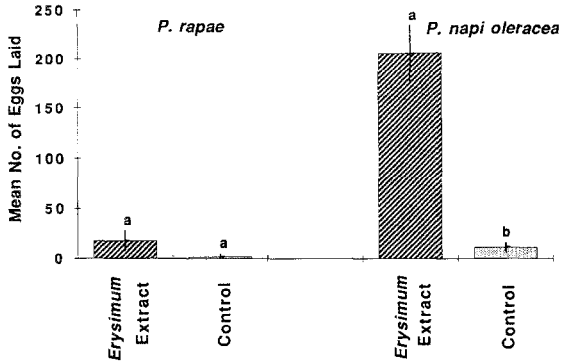


FIG. 1. Oviposition by *P. rapae* and *P. napi oleracea* on a choice of bean plants treated with postbutanol water extract of *E. cheiranthoides* or with 70% MeOH in H_2O (control). Five gram leaf equivalents were used for each replication. Replicated eight times. A replication consists of one bioassay cage with eight pairs of butterflies. Means (±SE) with the same letters in each series are not significantly different according to a paired *t* test ($P < 0.05$).

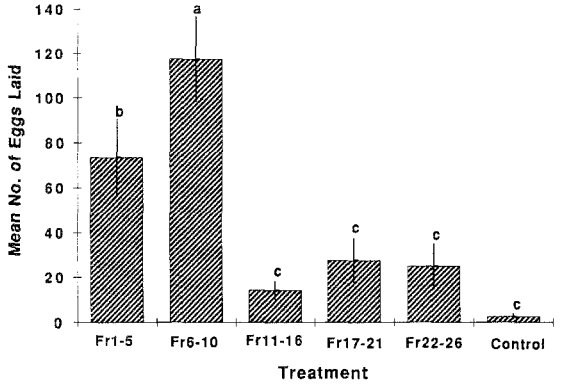


FIG. 2. Oviposition by *P. napi oleracea* on bean plants treated with different combinations of atmospheric-pressure column fractions from the postbutanol water extract of *E. cheiranthoides* or with 70% MeOH in H_2O (control). Five gram leaf equivalents were used for each replication. Replicated nine times. A replication consists of one bioassay cage with eight pairs of butterflies. Means (±SE) with the same letters are not significantly different according to a Waller-Duncan *K*-ratio *t* test ($K = 100$).

pared with the control (Figure 2). The results demonstrated that the most important stimulants were limited to fractions 1–10. These active fractions were therefore further tested in smaller combinations in subsequent bioassays. As shown in Figure 3, fractions 6–8 were the most stimulatory, and minor activity

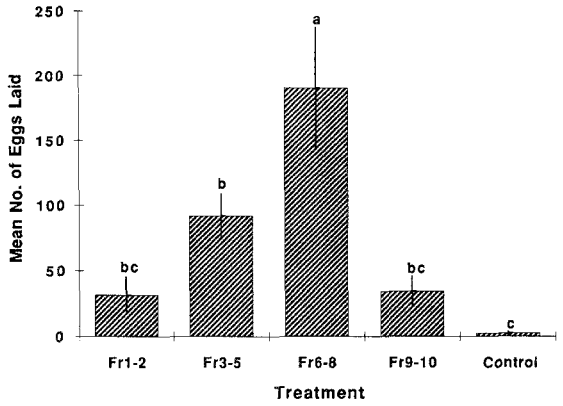


FIG. 3. Oviposition by *P. napi oleracea* on bean plants treated with combinations of selected atmospheric-pressure column fractions (1–10) from the postbutanol water extract of *E. cheiranthoides* or with 70% MeOH in H_2O (control). Five gram leaf equivalents were used for each replication. Replicated seven times. A replication consists of one bioassay cage with eight pairs of butterflies. Means (±SE) with the same letters are not significantly different according to a Waller-Duncan *K*-ratio *t* test ($K = 100$).

was detected from fractions 3–5. Fractions 1 and 2 or 9 and 10 were not significantly different from the control.

Each of these 10 fractions was subjected to glucosinolate analysis by HPLC of the desulfated products. Two glucosinolates were detected in fractions 5–8 from the postbutanol water extract, and the highest concentration of these glucosinolates was found in fractions 6 and 7 (Figure 4A). When total glucosinolates in *E. cheiranthoides* were analyzed in the same way, these two glucosinolates were the most abundant (Figure 4B). When fractions 6–8 were combined and developed on the preparative TLC plates, three prominent bands were individually collected. These compounds, with hR_fs of 10.0, 23.8 and 41.5, were named **1, 2,** and **3,** respectively. Desulfation foilowed by HPLC analyses of these compounds showed that **1** and **2** were glucosinolates (Figure 5A and B) and the retention times of their desulfated products were identical to those of the two compounds in the desulfated samples of the active fractions (Figure 4A). Compound **3** was not a glucosinolate. In bioassays using cabbage postbutanol water extracts (containing oviposition stimulants) as controls (Figure 6), significantly more eggs were laid by *P. napi oleracea* on bean plants treated with either **1** or **2** than on the control plants (OSI = 22.2 and 13.1, respectively), indicating that both compounds are highly stimulatory to the insects. Compound **3** was not active as a stimulant when compared with cabbage extract (OSI = −64.4).

The UV spectra of both compounds **1** and **2** were characteristic of aliphatic

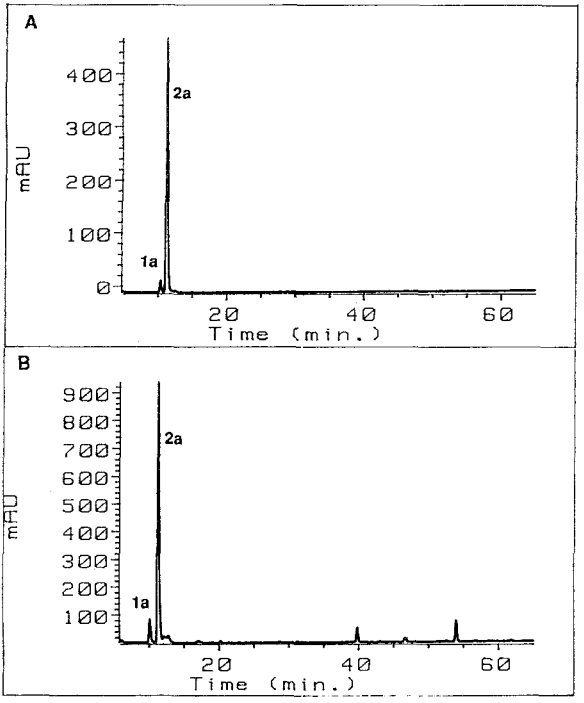


FIG. 4. HPLC separation of desulfoglucosinolates from: (A) the active open-column fractions (only fraction 7 is shown); (B) the postbutanol water extract of *E. cheiranthoides*. UV monitoring at 219 nm.

glucosinolates, with the maximum absorption at ca. 230 nm. The retention time on HPLC, and the hR_f on TLC of desulfated or nondesulfated compound **1** were identical to those of standard glucoiberin (Carl Roth, D75 Karlsruhe). Identification of **1** was further confirmed by ^{13}C NMR data of its desulfated product **1a** (Figure 7, Table 1).

Compound **2** was identified as glucocheirolin solely on the basis of ^{13}C NMR data of the desulfated product **2a** (Figure 7, Table 1). Products **1a** and **2a** are structurally related. The sulfinyl group in **1a** is replaced by a sulfonyl group in **2a**. In **2a**, the methyl carbon (C-4), which is attached to a sulfonyl group, resonates 2.5 ppm downfield compared to that of **1a**. A similar difference is observed in the ^{13}C NMR data of **1** and **2** reported by Cox et al. (1984).

Oviposition by both *P. napi oleracea* and *P. rapae* was stimulated by the commercial glucoiberin at a dose of 1 mg/plant, but the relative response of the two species differed dramatically when offered a choice of glucosinolate or cabbage extracts (Figure 8, OSI = 35.0 for *napi* and −57.7 for *rapae*).

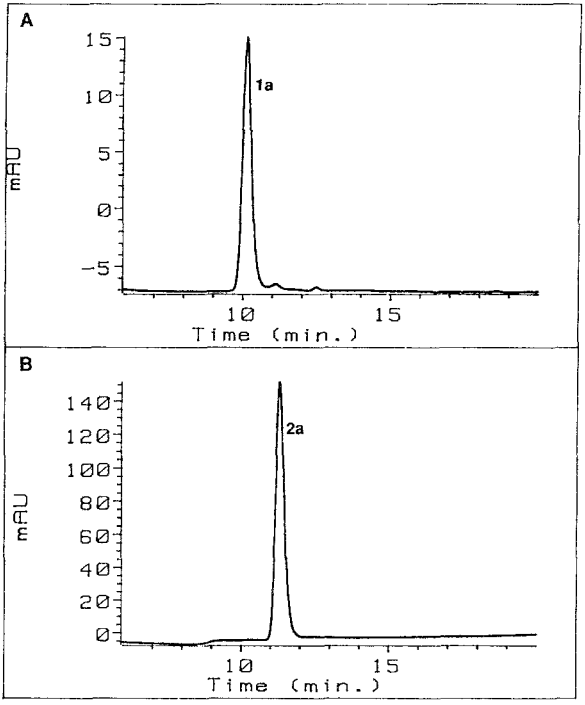


FIG. 5. HPLC of the desulfated products **1a** (A) and **2a** (B), respectively, from compounds **1** and **2** isolated by TLC of the active open-column fractions (Figure 4). UV monitoring at 219 nm.

To test the relative importance of these two glucosinolates in stimulating oviposition by either *Pieris* species, a bioassay was performed using the natural glucoiberin and glucocheirolin collected from *E. cheiranthoides* by TLC (Figure 9). The two compounds were equally stimulatory to *P. napi oleracea*, although the concentration of glucoiberin was about 10 times lower than that of glucocheirolin (as determined by HPLC, Figure 4). At the test concentration used, isolated glucoiberin did not stimulate oviposition by *P. rapae*. *P. rapae* laid more eggs on plants treated with glucocheirolin than on plants treated with solvent alone. However, the stimulatory effect of glucocheirolin on *P. rapae* was quite weak, based on the relatively low numbers of eggs laid in each cage. This observation was confirmed when glucocheirolin from *E. cheiranthoides* was compared with an equivalent amount of cabbage postbutanol water extract. The average number of eggs laid in each replication by *P. rapae* on the glucocheirolin-treated plant was 45, while that on the cabbage-treated plant was 217 (OSI $= -64.5$, paired t test $P < 0.038$). Furthermore, cabbage plants

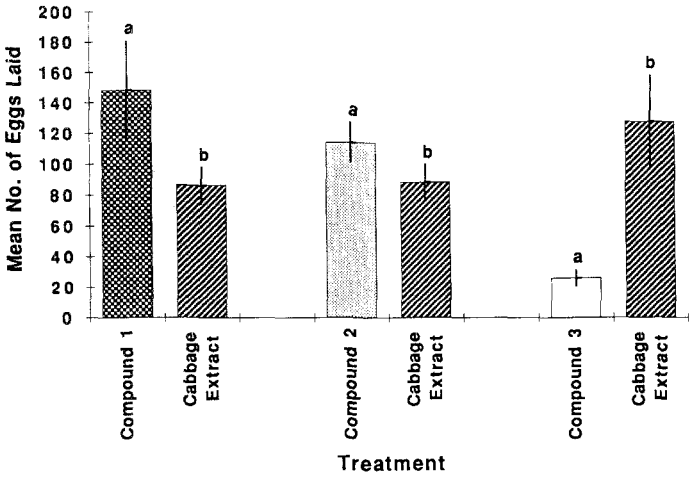
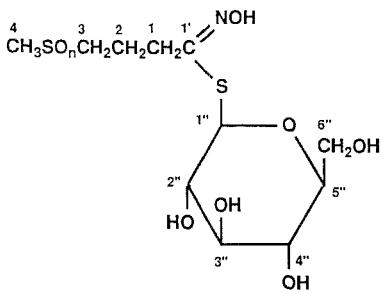


FIG. 6. Oviposition by *P. napi oleracea* on a choice of bean plants treated with compound **1**, **2**, or **3** isolated by TLC of the active atmospheric-pressure column fractions from the postbutanol water extract of *E. cheiranthoides*, or with postbutanol water extract of cabbage (control). Three gram leaf equivalents from each compound or extract were used for each replication. Replicated five times. A replication consists of one bioassay cage with eight pairs of butterflies. Means (±SE) with the same letters in each series are not significantly different according to a paired *t* test ($P < 0.05$).



1a. n=1

2a. n=2

FIG. 7. Structures of desulfated products **1a** and **2a** from compounds **1** and **2**, respectively.

TABLE 1. ^{13}C NMR DATA OF DESULFATED PRODUCTS **1a** AND **2a** FROM COMPOUNDS **1** AND **2**, RESPECTIVELY

Carbon	Chemical shift (ppm)	
	1a	**2a**
1	31.94	31.61
2	21.57	21.38
3	54.03	54.53
4	38.21	40.71
1′	152.86	152.09
1″	83.45	83.42
2″	74.63	74.60
3″	79.91	79.93
4″	71.51	71.45
5″	82.53	82.50
6″	62.90	62.84

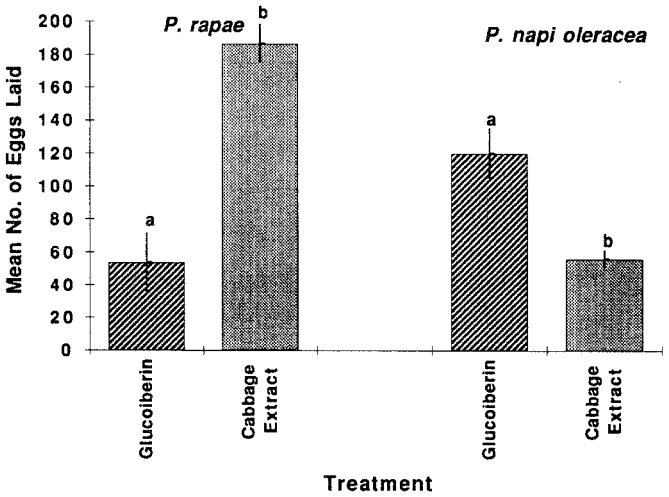


FIG. 8. Oviposition by *P. rapae* and *P. napi oleracea* on a choice of bean plants treated with commercial glucoiberin (1 mg/plant) or with postbutanol water extract of cabbage (control, 4 gram leaf equivalents/plant). Replicated four times. A replication consists of one bioassay cage with eight pairs of butterflies. Means ($\pm$SE) with the same letters in each series are not significantly different according to a paired t test ($P < 0.05$).

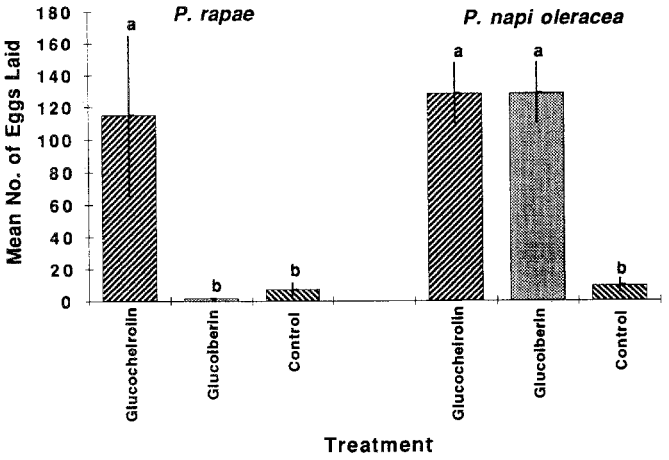


FIG. 9. Oviposition by *P. rapae* and *P. napi oleracea* in response to a choice of glucoiberin or glucocheirolin, collected by TLC of the active atmospheric-pressure column fractions from the postbutanol water extract of *E. cheiranthoides*. Three gram leaf equivalents from each compound were used for each replication. Control = 70% MeOH in H_2O. Replicated four times. A replication consists of one bioassay cage with eight pairs of butterflies. Means (±SE) with the same letters in each series are not significantly different according to a Waller-Duncan *K*-ratio *t* test ($K = 100$).

treated with the postbutanol water extract of *E. cheiranthoides* were much less preferred by *P. rapae* than those sprayed with solvent alone. An ODI of 45.8 (paired *t* test $P < 0.0118$) indicated the presence of deterrent(s) in the extract. HPLC confirmed that the extract contained a small amount of the known oviposition deterrents (cardenolides). This finding explains the fact that the postbutanol water extract was not stimulatory to *P. rapae*, although the equivalent amount of glucocheirolin isolated from it was active. The results indicate that glucoiberin and glucocheirolin can account for stimulation of oviposition by *P. napi oleracea* on *E. cheiranthoides*. Furthermore, glucocheirolin in this plant can stimulate oviposition by *P. rapae*, but the effect of the glucocheirolin is readily outweighed by the deterrents.

A previous study (Huang and Renwick, 1993) showed that the butanol extract of *E. cheiranthoides* was strongly deterrent to *P. rapae*, with an ODI of 91.5 (paired *t* test $P < 0.0009$), but it had little effect on *P. napi oleracea* (ODI = 17.2, paired *t* test $P < 0.26$). In this study, the combination of cardenolides (erysimoside, erychroside, and erycordin) isolated from the butanol extract by HPLC was highly deterrent to *P. rapae* (Figure 10, ODI = 75.4). However, *P. napi oleracea* was much less sensitive to these deterrents, although significantly fewer eggs were laid on test plants than on control plants (Figure

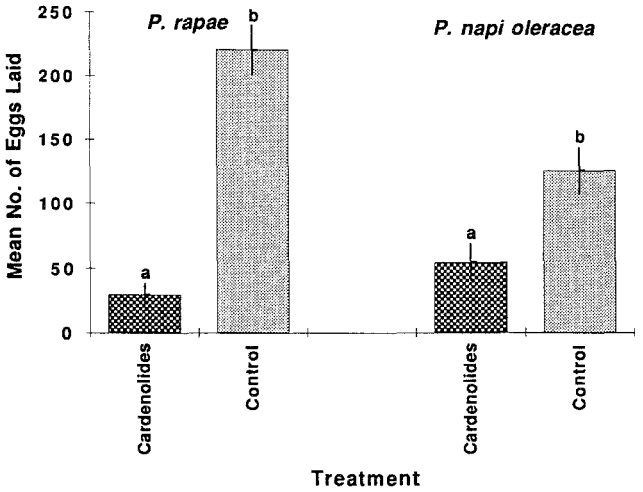


FIG. 10. Oviposition by *P. rapae* and *P. napi oleracea* on a choice of cabbage plants treated with cardenolides (erysimoside, erychroside, and erycordin) isolated by HPLC from the butanol extract of *E. cheiranthoides*, or with 80% MeOH in H_2O (control). Five gram leaf equivalents were used for each replication. Replicated eight times. A replication consists of one bioassay cage with eight pairs of butterflies. Means ($\pm$SE) with the same letters in each series are not significantly different according to a paired *t* test ($P < 0.05$).

10, ODI = 43.4). No additional deterrents to *P. napi oleracea* were found in the butanol extract. Instead, the remaining portion (the cardenolides had been removed) of the butanol extract was stimulatory to *P. napi oleracea*, and HPLC of the desulfated sample of this portion showed the presence of some glucocheirolin and glucoiberin, which had been partially extracted from the water into butanol. This explains why the deterrence of the whole butanol extract was not as strong as that of the cardenolides isolated from the extract.

DISCUSSION

Previous studies suggest that landing by ovipositing females of *P. rapae* is guided mainly by visual cues and that acceptance or avoidance is then controlled by contact chemoreception of semiochemicals on the plant surface (Traynier, 1979; Renwick and Radke, 1988). In this study we have confirmed and clarified the key role of plant chemistry in host-plant selection by both *P. rapae* and *P. napi oleracea*. Two different classes of plant secondary substances, glucosinolates and cardenolides, determine the acceptance or rejection of *E. cheiranthoides* by these two *Pieris* species. The isolation and identification

of glucoiberin and glucocheirolin as the most active stimulatory compounds in *E. cheiranthoides* clearly indicates that these glucosinolates can account for recognition of this plant as a suitable host for *P. napi oleracea*. Similarly, the cardenolides in *E. cheiranthoides* act as deterrents, which prevent *P. rapae* from ovipositing on this plant species.

The experimental procedures employed in this study have provided us with a way to explain the differential acceptance of *E. cheiranthoides* by the two *Pieris* species. The separation of stimulants from deterrents is based on their relative solubilities in water and butanol. However, this separation is obviously not absolute, and traces of opposing stimuli may have a distinct effect on the insect behavior. The small amounts of glucoiberin and glucocheirolin present in the butanol fraction from *E. cheiranthoides* were sufficient to weaken the deterrent effect of the cardenolides on *P. napi oleracea*. In the case of *P. rapae*, the slight stimulatory effect of glucocheirolin was outweighed by the deterrent effect of traces of cardenolides in the postbutanol water extract. These observations point to the possibility of a delicate balance of positive and negative stimuli affecting the insect behavior.

P. rapae and *P. napi oleracea* are specialists whose host ranges are restricted to members of the Cruciferae and a few related plant families that contain glucosinolates (Verschaffelt, 1911). Glucosinolates have been shown to be important larval feeding stimulants (Hovanitz and Chang, 1963) or oviposition stimulants for *Pieris brassicae* (van Loon et al., 1992), *P. napi mocdunnoughii* (Rodman and Chew, 1980), and several other crucifer specialists (reviewed by Schoonhoven, 1972). However, the idea that glucosinolates are responsible for host recognition by all crucifer-feeding insects has been challenged (Nielsen, 1978; Chew, 1988). In this study, we find that the presence of glucosinolates does not always result in the acceptance of a crucifer by a *Pieris* species. Stimulatory effects of glucosinolates on *Pieris* butterflies may differ among insect species or among chemical classes of the glucosinolates on a particular insect species. For example, *P. rapae* refuses to oviposit on *E. cheiranthoides* despite the presence of large amounts of glucocheirolin and some other glucosinolates including glucoiberin. The aliphatic glucocheirolin from *E. cheiranthoides* was only weakly stimulatory to *P. rapae*, but oviposition of this insect species is strongly stimulated by the indole glucosinolate, glucobrassicin, in cabbage plants (Traynier and Truscott, 1991; Renwick et al., 1993). On the other hand, *P. napi oleracea* prefers the aliphatic glucosinolates in *E. cheiranthoides* over the indole glucosinolate in cabbage (Figures 6 and 8). *P. napi oleracea* laid many more eggs on bean plants treated with commercial glucoiberin (1 mg/plant) than on bean plants treated with cabbage extract (4 gram leaf equivalents/plant). Because cabbage plants contain the indole glucosinolate at an average of 50 mg/100 g (reviewed by van Loon et al., 1992), it is safe to conclude that the amount of glucobrassicin used in this bioassay was equal to or more than that of glu-

coiberin. Thus, glucoiberin is more stimulatory to *P. napi oleracea* than is the indole glucosinolate. These results indicate that differential sensitivities to glucosinolates have evolved in the indigenous *P. napi oleracea* and the naturalized *P. rapae*.

The structural requirements for activity of glucosinolates may be quite complex. The two major glucosinolates in *E. cheiranthoides* are closely related structurally, but *P. napi oleracea* is apparently more sensitive to glucoiberin than to glucocheirolin. The response of this species to the natural concentration of the two glucosinolates was approximately equal, but glucocheirolin is present at about 10 times the level of glucoiberin.

Chemical deterrents may frequently co-occur with stimulants in a potential host plant. The final step of rejection or acceptance of *E. cheiranthoides* by ovipositing *P. rapae* or *P. napi oleracea* is mediated by more than one chemical stimulus in the plant. *E. cheiranthoides* is highly preferred by *P. napi oleracea*, although cardenolides (including erysimoside and erychroside) in the plant are, to a certain extent, deterrent to this insect. In contrast, *P. rapae* avoids *E. cheiranthoides* as a host, even though the glucosinolates in the plant are somewhat stimulatory. These results suggest that the relative importance of the stimulants and the deterrents in host-plant recognition by the two *Pieris* species may differ considerably. In *P. napi oleracea*, the negative effect is outweighed by the positive effects, leading to acceptance of *E. cheiranthoides*. However, in *P. rapae*, the minor stimulatory activity of glucosinolates is masked by the potent deterrent activity of the cardenolides, resulting in rejection of the plant. The significance of such a balance between negative and positive chemical input in host selection by herbivorous insects (Dethier, 1982; Miller and Strickler, 1984; Renwick and Radke, 1987) is clearly demonstrated.

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