A Flavonoid, Luteolin-7-Glucoside, as well as Salicin and Populin, Stimulating the Feeding of Leaf Beetles Attacking Salicaceous Plants¹

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Luteolin-7-glucoside was isolated as a feeding stimulant to leaf beetles, *Chrysomela vigintipunctata costella* and *Plagioderia versicolora distincta* in addition to salicin from the leaves of *Salix gracilistyla*. Feeding response of three species of Salicaceae-feeding leaf beetles, *C. v. costella*, *P. v. distincta* and *Lochmaea capreae cribrata*, to the three chemicals, luteolin-7-glucoside, salicin and a salicin derivative, populin, varied species-specifically. Salicin and populin stimulated the feeding in *C. v. costella* and *P. v. distincta* but not in *L. c. cribrata*, while all three species responded to luteolin-7-glucoside.

Salicin was found in all *Salix* species examined, and populin was always accompanied by salicin in the genus *Populus*. Luteolin-7-glucoside was also distributed in Salicaceae. These results suggest that the host specificity of *C. v. costella* and *P. v. distincta* depends on the presence of salicin and populin as well as luteolin-7-glucoside. On the other hand, the cue substance in *L. c. cribrata* seems to be flavonoids such as luteolin-7-glucoside.

INTRODUCTION

Salicaceous plants are known to contain the characteristic constituents, salicin and populin. Salicin has been reported as a feeding stimulant in Salicaceae-feeding insects, *Phyllodecta vitellinae* (HUTCHINSON, 1931) and *Plagioderia versicolora distincta* (MATSUMOTO, 1978), but the effect of populin has not been studied in these insects. These chemicals have been supposed to be the chemical cues in the host selection of Salicaceae-feeding insects.

To clarify this presumption, a series of experiments was carried out using three species of leaf beetles, *Chrysomela vigintipunctata costella* (MARSEUL), *Plagioderia versicolora distincta* BALY and *Lochmaea capreae cribrata* SOLOSKY which feed on salicaceous plants.

First, feeding stimulants including salicin and populin were isolated from salicaceous plant leaves. Then, the action of the isolated chemicals in the feeding of the leaf beetles was tested. Finally, the distribution of these chemicals was examined in various species of salicaceous plants.

MATERIALS AND METHODS

Insects. *Chrysomela vigintipunctata costella* used in the experiments were caught at the

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Salicin

Populin

Fig. 1. Chemical structures of salicin and populin.

Fig. 2. Scheme of isolation of the feeding stimulants.

larval stage on *Salix babylonica* L. f. *Rokkaku* and reared on the same plant. *Plagiodera versicolora distincta* were selected from the laboratory culture maintained on *S. babylonica* L. f. *Rokkaku*. *Lochmaea capreae cribrata* were gathered at the adult stage on *S. gracilistyla* and reared on the same plant.

Chemicals. Salicin (Fig. 1) was obtained from Wako Pure Chemical Industries, Ltd. Populin (Fig. 1) was synthesized by the method of RICHMYERE and YEAKEL (1934). Luteolin-7-glucoside was isolated from *S. Giligiana* using the method of MORITA et al. (1974). The purities of populin and luteolin-7-glucoside were checked by thin layer chromatography.

Bioassay. The effects of respective substances on the feeding of leaf beetles were examined in a choice trial test. Twenty adult beetles starved for 24 hr were released into a plastic petri dish (9 cm in dia. and 1.5 cm deep) in which four pieces of filter paper (2×2 cm, 2: treated, 2: control) were placed. The degree of nibbling by the insects was compared between the pieces moistened with test chemical solution and

those with control solution 24 hr after the release. The feeding response was judged from the differences between test and control pieces, and degree of feeding stimulation was expressed by the number of '+' marks. The mark '±' was used to indicate no stimulative effect. All assays were conducted in complete darkness at 24–25°C. Comparisons were made at 3 concentration levels of 0.1, 0.01 and 0.001 M with 5 replications each. Details of the testing methods were described in a previous paper (MATSUDA, 1976).

Isolation of feeding stimulants from S. gracilistyla. In our investigation, the plant most favored by *C. v. costella* and *L. c. cribrata* was *S. gracilistyla* (MATSUDA and MATSUO, 1983). *S. gracilistyla* fresh leaves, therefore, were used as the isolation materials of the feeding stimulant. The isolation procedure is summarized in Fig. 2.

Detection of salicin, populin and luteolin-7-glucoside. The plants used in the chemical analysis were as follows: willow, *Salix babylonica* L. f. *Rokkaku*, *S. Bakko*, *S. chaenomeloides*, *S. daiseniensis*, *S. Giligiana*, *S. integra*, *S. koriyanagi*, *S. Matsudana* K. *tortuosa*, *S. sachalinensis*, *S. vulpina*, poplar, *Populus Maximowiczii*, *P. nigra* var *italica* and *P. Sieboldii*. These mature leaf samples were collected in the Botanical Garden of Tohoku University, Sendai.

Fifty grams of finely chopped leaves was extracted with 500 ml of methanol for 24 hr and the extracts were filtered. The extraction was repeated two more times. After evaporation of the combined extracts under vacuum, the obtained syrup was dissolved in 100 ml of water and filtered. The filtrate was washed with chloroform twice and extracted with 200 ml of ethyl acetate several times. The combined extracts were evaporated under vacuum and the residue was dissolved in a minimum amount of methanol. The crude ethyl acetate fraction and methanol extract were examined by silicagel thin layer chromatography with salicin and populin as the control. Solvent systems used were benzene-methanol (30:70) and isopropanol-water (85:15). The salicin and populin were detected as a grey spot by spraying anisaldehyde-sulphuric reagent.

The detection of luteolin-7-glucoside from 13 species of salicaceous plants was carried out by the methods shown in Fig. 2. The ethyl acetate fraction obtained from salicaceous plant leaves (100 g) was concentrated under vacuum and the obtained solution was allowed to stand overnight. The resulting precipitates were recrystallized from pyridin-methanol (3:1). The CMR spectra of this compound were compared with that of authentic luteolin-7-glucoside.

RESULTS

Isolation of feeding stimulant from S. gracilistyla

The precipitates obtained from *S. gracilistyla* leaves (500 g) were recrystallized from pyridin-methanol (3:1); the yield was 210 mg. The isolated substances gave yellow shining needles, m.p. 254–256°C. The needles were colored dark brown by FeCl₃, reddish violet by HCl-Zn and orange by HCl-Mg. The spectra of CMR (Fig. 3) and UV of this compound agreed with that of authentic luteolin-7-glucoside. The spectra of CMR of this compound coincided with the analytical data of luteolin-7-glucoside (AGRAWAL and RASTOGI, 1981; MARKHAM et al., 1978). This compound was therefore concluded to be a flavonoid, luteolin-7-glucoside.

Ten milligrams of the flavonoid was hydrolyzed by refluxing for 2 hr at 100°C with 5% HCl. Yellow precipitates were collected by filtration, washed with water, dried

Fig. 3. CMR spectra of luteolin-7-glucoside.

Table 1. Effects of salicin, populin and luteolin-7-glucoside on the feeding of Salicaceae-feeding leaf beetles

Compound	Feeding response		
	<i>C. v. costella</i>	<i>P. v. distincta</i>	<i>L. c. cribrata</i>
Salicin			
0.1 M : H ₂ O	±	±	±
0.01 M : H ₂ O	±	±	±
0.001 M : H ₂ O	±	±	±
0.1 M in 0.1 M sucrose : 0.1 M sucrose	++	++	±
0.01 M in 0.1 M sucrose : 0.1 M sucrose	++	++	±
0.001 M in 0.1 M sucrose : 0.1 M sucrose	+	+	±
Populin			
0.1 M : H ₂ O	++	±	±
0.01 M : H ₂ O	++	±	±
0.001 M : H ₂ O	+	±	±
0.1 M in 0.1 M sucrose : 0.1 M sucrose	++	+	±
0.01 M in 0.1 M sucrose : 0.1 M sucrose	++	+	±
0.001 M in 0.1 M sucrose : 0.1 M sucrose	+	±	±
Luteolin-7-glucoside			
0.1 M : H ₂ O	++	±	+
0.01 M : H ₂ O	+	±	+
0.001 M : H ₂ O	±	±	±
0.1 M in 0.1 M sucrose : 0.1 M sucrose	++	++	++
0.01 M in 0.1 M sucrose : 0.1 M sucrose	+	+	++
0.001 M in 0.1 M sucrose : 0.1 M sucrose	±	±	±

and weighed (6.0 mg). The identification of the isolated aglycon was confirmed by paper chromatography with two solvent systems: *n*-butanol-acetic acid-water (4: 1: 2); acetic acid-water (60: 40). *R_f* values agreed with those of authentic luteolin in both of the solvent systems tested. The filtrate in the above experiments was concentrated under vacuum, and dried over NaOH. The sugar was identified by paper chromatography as glucose based on the comparison of *R_f* values with those of authentic chemicals. The ratio of aglycon to the glucoside in flavonoid was estimated to be 60%, which indicates the presence of 1 mole of sugar per mole of aglycon. These results also proved that this compound was luteolin-7-glucoside.

The precipitate from Fr. 4 was recrystallized from water. Two dimensional paper chromatography analysis of this compound was carried out with *n*-butanol-acetic acid-water (4: 1: 5) and 6% acetic acid. The *R_f* values agreed with authentic salicin.

Feeding response of three leaf beetle species to the chemicals

The effects of salicin, populin and luteolin-7-glucoside on the feeding of three leaf beetle species are summarized in Table 1. The feeding of *C. v. costella* was stimulated by salicin and populin at 0.001, 0.01 and 0.1 M in combination with 0.1 M sucrose. Populin was also effective for this insect even without sucrose. *P. v. distincta* was stimulated to feed by salicin at 0.001, 0.01 and 0.1 M, and by populin at 0.01 and 0.1 M in the presence of sucrose. The feeding of *L. c. cribrata* was not stimulated by these compounds at any concentration level tested with or without sucrose.

The feeding of *L. c. cribrata* was stimulated by luteolin-7-glucoside at 0.01 and 0.1 M with or without sucrose. *C. v. costella* and *P. v. distincta* were stimulated to feed by this compound as well as salicin and populin at 0.01 and 0.1 M in the presence of sucrose. The former was stimulated without sucrose. Luteolin had no stimulation at 0.001–0.1 M concentration in the three species.

Detection of salicin, populin and luteolin-7-glucoside in salicaceous plants leaves

The results are shown in Table 2. Salicin, populin and flavonoids known in the leaves of salicaceous plants are also listed in Table 2. Salicin was detected in all salicaceous plant leaves tried and populin in *P. Maximowiczii*, *P. nigra* var *italica* and *P. Sieboldii*. From the literature, salicin occurs in *S. eriocataphylla*, *S. fragaris*, *S. gracilistylodes*, *S. myrsinifolia*, *S. pentandra*, *S. purpurea*, *S. repense*, *P. alba*, *P. balsamifera*, *P. candicans*, *P. grandidentata*, *P. tremula*, *P. tremuloides*, *P. trichocarpa*, *C. arbutifolia* and *T. urbaniana*, and populin also in *P. balsamifera*, *P. grandidentata*, *P. tremula*, *P. tremuloides* and *P. trichocarpa*.

Luteolin-7-glucoside was detected from *S. daiseniensis* and *S. vulpina*. From the literature, this compound is present in *S. Giligiana*, *S. gymnolepis*, *S. purpurea* and *P. candicans*.

DISCUSSION

Salicaceous plants are known to contain salicin and populin as their characteristic plant substances. The former compound was found in the *Salix* and *Populus* species examined here and the latter was contained in *Populus* species (Table 2). Table 2 also shows the wide distribution of these chemicals among the plant species of the family Salicaceae.

Salicin has been reported to deter the feeding of herbivorous insects. For example, in two lepidopterous larvae, the African armyworm, *Spodoptera exempta* (CLARK, 1981)

Table 2. Distribution of salicin, populin and luteolin-7-glucoside in the leaves of salicaceous plants

Scientific Name	Salicin	Populin	References	Flavonoids	References
<i>Salix</i>					
<i>Salix alba</i>					
<i>S. babylonica</i>	○	—	in this article	Rhamnazin-3-glucoside	THIEME, 1968
<i>L. f. Rektaku</i>	○	—	"	Apigenin 7-(4'- <i>p</i> -coumaroyl)-glucoside	KARL et al., 1976
<i>S. Bakko</i>					
<i>S. caesia</i>	○	—	in this article	Lu-7-glucosylarabinoside	MORITA et al., 1976
<i>S. chaenomeloides</i>	○	—	"	Lu-7-xylosylarabinoside	HARBORNE, 1975
<i>S. daisei</i>	○	—	THIEME, 1965	Lu-7-glucoside	in this article
<i>S. eriocataphylla</i>	○	—	"		
<i>S. fragaris</i>	○	—	in this article	Lu-7-glucoside	MORITA et al., 1974
<i>S. Giliigiana</i>	○	—	"	"	in this article
<i>S. gracilistyla</i>	○	—	HIRAO, 1954	"	FUJIKAWA and NAKAHARA, 1948
<i>S. gracilistyloloides</i>	○	—	in this article		
<i>S. gymnolepis</i>	○	—	"		
<i>S. integra</i>	○	—	in this article		
<i>S. koriyanagi</i>	○	—	"		
<i>S. Matsudana</i>	○	—	"		
<i>K. tortuosa</i>	○	—	THIEME, 1965	Lu-7-glucuronide	MORITA et al., 1974
<i>S. myrsinifolia</i>	○	—	"	Lu-3'-methylether 7-glucuronide	
<i>S. pentandra</i>	○	—	"		
<i>S. purpurea</i>	○	—	"	Lu-7-glucoside	JARRET and WILLIAMS, 1967
				Narigenin-7-glucoside	
				Eiodictyol-7-glucoside	
<i>S. repens</i>	○	—	"		

<i>S. sachalinensis</i>	○	—	in this article			
<i>S. triandra</i>						HARBORNE, 1967
<i>S. viminalis</i>						KARL et al., 1977
<i>S. vulpina</i>	○	—	in this article	Qu-3-rutinoside Isorhamnetin-3-(6'-acetyl- glucoside)		in this article
Populus				Lu-7-glucoside		
<i>Populus alba</i>	○		THIEME, 1967			
<i>P. balsamifera</i>	○	○	PEARL and DARLING, 1968			
<i>P. candicans</i>	○		THIEME, 1967	Lu-7-glucoside		THIEME, 1969
				Myricetin-3-galactoside		
<i>P. grandidentata</i>	○	○	PEARL et al., 1962	Qu-3-glucoside		
<i>P. Maximowiczii</i>	○	○	in this article	Qu-3-glucuronide		PEARL and DARLING, 1963
<i>P. nigra</i> var <i>italica</i>	○	○	"			
<i>P. Sieboldii</i>	○	○	"			
<i>P. tremula</i>	○	○	MIYAMOTO and OKAWA, 1942; THIEME, 1967			
<i>P. tremuloides</i>	○	○	PEARL et al., 1962; THIEME, 1967			
<i>P. trichocarpa</i>	○	○	THIEME, 1967			
<i>Chosenia</i>						
<i>Chosenia arbutifolia</i>	○		HIRAO, 1954			
<i>Toisusu</i>						
<i>Toisusu urbaniana</i>	○		HIRAO, 1954			

○ sign is used to indicate the presence of salicin or populin. Lu: luteolin, Qu: quercetin.
 — sign indicates that populin could not be detected from the samples.

and the tobacco hornworm, *Manduca sexta* (SCHOONHOVEN, 1969), salicin acted as a feeding deterrent. It seems that salicin and populin have a defensive role against the attack of insects in salicaceous plants.

To the contrary, both chemicals stimulated feeding of *C. v. costella* and *P. v. distincta* whose host range is restricted to salicaceous plants. These leaf beetles seem to have overcome the toxic effects of the chemicals and to actually utilize the chemicals as a guiding substance to host plants.

The feeding of *L. c. cribrata*, *C. v. costella* and *P. v. distincta* was also stimulated by luteolin-7-glucoside. The distribution of luteolin glycosides in the leaves of salicaceous plants is shown in Table 2. *S. Giligiana*, *S. gymnolepis*, *S. purpurea* and *P. candicans* contain luteolin-7-glucoside and this compound was identified in *S. daisei*, *S. gracilistylla* and *S. vulpina* in the present experiments. Luteolin-7-glucoside is also one of the characteristic components in salicaceous plants, but this chemical is distributed in many botanically unrelated plants as well (HARBORNE et al., 1975).

L. c. cribrata did not respond to salicin and populin but did to luteolin-7-glucoside. Host range of this species is not restricted to salicaceous plants. It can feed on *Lyschiton camtschatense* of family araceae in the field. From these facts, it can be suggested that flavonoids in the plant attract a wider host range of leaf beetles and more specific ranges are restricted by characteristic substances to narrow phylogenetic groups of plant, such as salicin and populin.

A similar example of host selection was reported in the leaf beetles attacking polygonaceous plants. Four species of leaf beetles, *Gastrophysa atrocyanea*, *Gallerucida bifasciata*, *Gallerucida nigromaculata* and *Galerucella vittaticollis*, were stimulated to feed by flavonoids, quercitrin and rutin which were contained in polygonaceous plants (MATSUDA, 1976). The former three species, whose host ranges were restricted to polygonaceous plants, responded to organic acids, such as oxalic acid, characteristic to these plants (MATSUDA and MATSUMOTO, 1975). *G. vittaticollis*, on the other hand, did not respond to the organic acids; this species feeds on strawberry as well as polygonaceous plants.

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