（様式５）(Style5)

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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Name

学位論文題目：
Title of Dissertation

Studies on host recognition substances for sucking rice pests in the rice plant, *Oryza sativa* L.
（イネに含まれる吸汁性昆虫の寄主認識物質に関する研究）

学位論文要約：
Dissertation Summary

Rice planthoppers and leafhoppers are very notorious pests of rice plant in Asian countries. They damage plants by sucking the sap and by plugging xylem and phloem with their feeding sheaths. Except direct feeding damage, rice planthoppers and leafhoppers are vectors of most of the currently known rice virus diseases. For instance, the green rice leafhopper, *N. nigropictus* can transmit rice dwarf virus (RDV), rice tungro virus, rice transitory yellowing virus, rice yellow dwarf mycoplasma-like organisms (De Datta S. K., 1981; Nasu S., 1963) and even be capable of transovarially transmitting RDV to its progeny (Nasu S., *et. al.*, 1967). On the other hand, the white-backed planthopper, *S. furcifera* is the vector for the entomophilous fungus, *Erynia delphacis* (Entomophthorales) (Matsui T., *et. al.*, 1998) and the rice black-streaked dwarf virus is transmitted by it(Zhou G. H., *et. al.*, 2013). Although chemical insecticides, natural enemies and high resistant rice are widely applied to control these pests, the environmental pollution and insect resistances become extremely difficult problems in the world. Therefore, a new pest control method based on the feeding behaviors of planthoppers and leafhoppers is considered in this study.

The feeding behavior of sucking rice pests is well known to be classified into two phases: probing and sucking phases, which are controlled by physical and chemical factors in plants (Sō gawa K., 1974; Sō gawa K., 1982). In view of the precious studies, the probing stimulants in the rice plant for sucking rice pests have already been partially studied. Eight *C*-glycosylflavones were isolated from rice plant as partial probing stimulants towards the brown planthopper, *Nilaparvata lugens* (Kim M., *et. al.*, 1985; Besson E., *et. al.*, 1985). The probing stimulants for the smaller brown planthopper, *Laodelphax striatellus* (Francis A. A.,

et. al., 2000[b]) and *S. furcifera* (Francis A. A., *et. al.*, 2000[a]) also only were partially reported. The leafhoppers including *Nephotettix virescens*, *N. cincticeps* were studied by Takemura M.. (Takemura M., 2000). The *N. nigropictus*, however, has scarcely been researched about probing stimulants. Therefore, the study of *N. nigropictus* and *S. furcifera* were further undertaken in this study.

Rice plant (cv. Toyonishiki) was extracted three times with 90% methanol in water for 3 days and the extract was defatted three times with hexane to obtain crude rice extract. It was adjusted to a 2 g of fresh leaf and stem equivalent (eq)/ml concentration of the crude rice extract. When two insects of *N. nigropictus* and *S. furcifera*, were fed on 2 g eq/ml crude rice extract through parafilm membrane, many stylet sheaths were deposited on the parafilm membrane. The results indicated that crude rice extract had high probing activities for these two species. This extract was further loaded on an medium-pressure ODS column and eluted in sequence with methanol and water solutions to get ODS water fraction, ODS 20% methanol in water fraction, ODS 40% methanol in water fraction and ODS 100% methanol fraction. Both two species insects showed most active on ODS 40% methanol in water fraction among four fractions. However, when further bioassays were conducted on the individual fractions separated by preparative HPLC as well as their various combinations from the ODS 40% fraction, two species insects showed significantly different responses. Among of them, four peaks (**A**, **7**, **8-1**, and **8-2**) or any combination without one of the four peaks caused weak probing responses for *N. nigropictus*. Only when they were combined, the similar activities were recovered to ODS 40% methanol in water fraction for *N. nigropictus*. Similarly, for *S. furcifera*, only when four peaks (**5-2**, **5-3**, **7**, and **9-1**) were combine, the probing activitity showed higher than ODS 40% methanol in water fraction. The probing stimulants for *N. nigropictus* were determined as peak **A** (isoscoparin 2″ -*O*-glucoside), peak **7** {isoscoparin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside}, peak **8-1** {isoscoparin 2″ -*O*-(6‴ -(E)-*p*-coumaroyl)glucoside} and peak **8-2** {isovitexin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside}, using LC-MS and NMR spectra, respectively, as shown in Fig. 1. On the other hand, three special *C*-glycosylflavonoids; **5-2** {isoorientin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside}, **5-3** {isoorientin 2″ -*O*-(6‴ -(*E*)-*p*-coumaroyl)glucoside}, **7**, and **9-1** (tricin 5-*O*-glucoside) were determined as probing stimulants for *S. furcifera* in the rice plant, as shown in Fig. 2.

As an oligophagous insect, the *N. nigropictus* requires one common and three specific *C*-glycosylflavonoids as probing stimulants. The *S. furcifera*, another oligophagous insect, also needed four probing stimulants, including one common *O*-glycosylflavonoid, and three specific *C*-glycosylflavonoids. For the monophagous *N. lugens*, eight probing stimulants were mainly reported from the rice plant (Kim M., *et. al.*, 1985; Besson E., *et. al.*, 1985). This shows that in order for it to specifically select its host, the *N. lugens* requires many specific compounds as probing stimulants. The oligophagous rice pests *N. nigropictus* and *S. furcifera* did not need many specific flavonoid glycosides as probing stimulants in order to find their host plants, because they have dozens or several dozens of host plants. Therefore, it is considered that there are some relationships between the quality and quantity of probing stimulants for sucking rice pests and their host ranges. This hypothesis may support the idea that the probing stimulants might play a significant role in host recognition. Moreover, since the probing behavior is performed prior to the sucking behavior (Sogawa K., *et. al.*, 1982), manipulating the probing stimulants could become a new method to control sucking rice pests.

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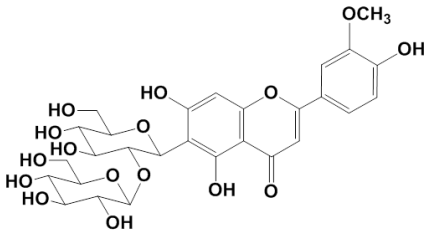
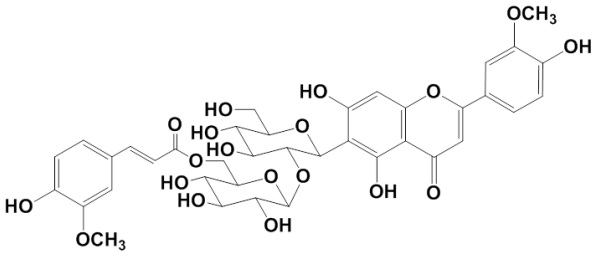
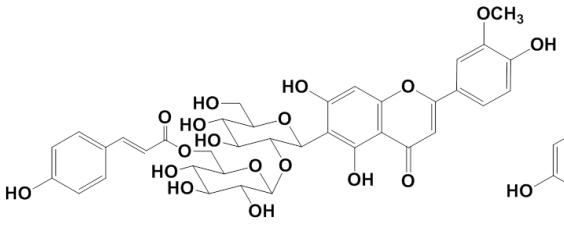


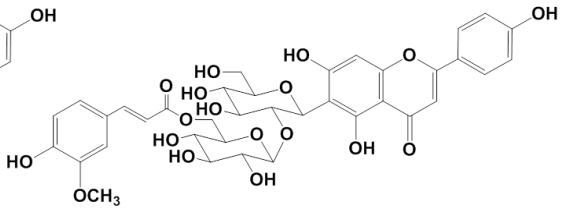
pkA isoscoparin 2″ -*O*-glucoside



pk7 isoscoparin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside

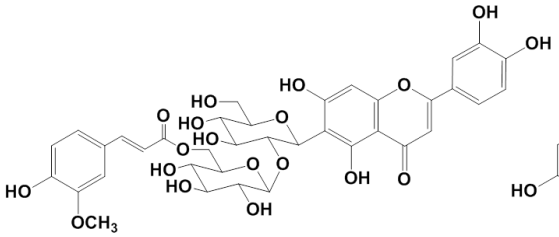


pk8-1 isoscoparin 2″ -*O*-(6‴ -(E)-*p*-coumaroyl)glucoside

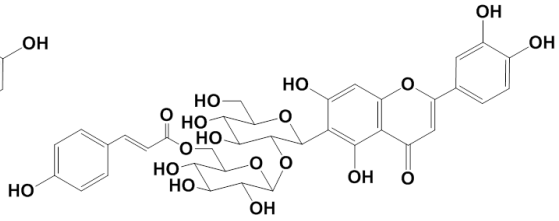


pk8-2 isovitexin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside

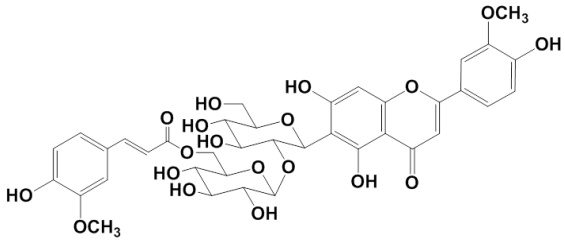
Fig. 1. The structures of probing stimulants for *N. nigropictus*



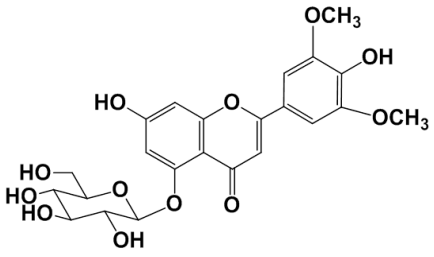
pk5-2 isoorientin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside



pk5-3 isoorientin 2″ -*O*-(6‴ -(*E*)-*p*-coumaroyl)glucoside



pk7 isoscoparin 2″ -*O*-(6‴ -(*E*)-feruloyl)glucoside



pk9-1 tricin 5-*O*-glucoside

Fig. 2. The structures of probing stimulants for *S. furcifera*

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Table 1. ^{1}H NMR Data (Coupling Constant *J*/Hz in Parentheses) of peaks **A**, **5-2**, **5-3**, **7**, **8-1**, **8-2**, and **9-1**

Proton	Chemical shift δ in ppm						
	A	**5-2**	**5-3**	**7**	**8-1**	**8-2**	**9-1**
3	6.73 s	6.37 m	6.36 s	6.55 br.s	6.57 br.s	6.45 s	6.79 d (2.0)
6	-	-	-	-	-	-	6.77 m
8	6.40 br.s	6.37 m	6.34 s	6.40 s	6.40 s	6.36 s	6.77 m
2'	7.51 m	7.30 d (2.0)	7.28 d (1.7)	7.38 br.s	7.38 br.s	7.71 d (9.0)	7.24 d (2.0)
3'	-	-	-	-	-	6.85 d (9.0)	-
5'	6.94 d (9.0)	6.83 d (8.5)	6.82 d (8.5)	6.86 m	6.87 d (8.0)	6.85 d (9.0)	-
6'	7.51 m	7.22 m	7.23 dd (8.5, 1.5)	7.36 m	7.37 dd (8.0, 2.0)	7.71 d (9.0)	7.24 d (2.0)
2''	-	7.10 br.s	7.31 d (8.3)	7.06 br.s	7.29 d (8.0)	7.09 br.s	-
3''	-		6.75 d (8.5)	-	6.76 d (8.0)	-	-
5''	-	6.77 d (8.0)	6.75 d (8.5)	6.75 d (8.0)	6.76 d (8.0)	6.77 d (8.0)	-
6''	-	6.90 d (7.5)	7.31 d (8.3)	6.86 m	7.29 d (8.0)	6.89 m	-
α	-	6.16 d (15.8)	6.07 d (16.0)	6.14 d (16.3)	6.08 d (16.0)	6.15 d (16.0)	-
β	-	7.21 d (15.8)	7.21 d (16.0)	7.17 d (16.3)	7.20 d (16.0)	7.19 d (16.0)	-
G-1	4.70 d (10.0)	4.71 d (9.5)	4.68 d (10.0)	4.72 d (9.5)	4.72 d (10.0)	4.72 d (10.0)	4.67 d (7.5)
G-2	4.30 d (9.3)	4.35 br.s	4.32 br.s	4.35 br.s	4.34 br.s	4.37 br.s	3.10~3.44 m
G-3	3.46 m	3.48 m	3.44 m	3.47 m	3.47 m	3.48 m	3.10~3.44 m
G-4	3.22 m	3.22 m	3.19 m	3.22 m	3.23 m	3.23 m	3.10~3.44 m
G-5	3.22 m	3.22 m	3.19 m	3.22 m	3.22 m	3.23 m	3.10~3.44 m
G-6a	3.46 m	3.48 m	3.42 m	3.47 m	3.44 m	3.46 m	3.52 m
G-6b	3.69 d (11.5)	3.68 dd (12.0,1.5)	3.67 dd (11.8, 1.7)	3.70 dd (11.5, 1.5)	3.70 dd (11.5, 1.9)	3.69 dd (11.9, 2.0)	3.71 d (10.0)
G-1'	4.23 d (7.5)	4.28 d (8.0)	4.25 d (8.0)	4.29 d (7.5)	4.28 d (8.0)	4.29 d (7.5)	-
G-2'	2.89 t (9.0)	2.95 t (8.3)	2.93 m	2.94 t (8.3)	2.95 m	2.96 m	-
G-3'	3.09 m	3.13 m	3.07 m	3.12 t (8.8)	3.10 m	3.12 m	-
G-4'	2.99 m	3.07 t (9.0)	3.02 m	3.06 t (8.8)	3.01 m	3.03 m	-
G-5'	2.72 t (7.5)	3.01m	3.00 m	3.01 m	3.01 m	3.01 m	-
G-6'a	3.18 m	3.73 m	3.71 m	3.70 dd (11.5, 1.5)	3.73 m	3.74 m	-
G-6'b	3.18 m	3.90 dd (11.5, 2.5)	3.84 dd (11.5, 3.0)	3.93 dd (11.0, 2.5)	3.90 dd (11.5, 2.5)	3.91 dd (11.8, 3.3)	-
5-OH	13.55 s	13.54	13.53 s	13.53 s	13.52 s	13.52 s	-
-OCH$_3$	3.89 s	3.82 s	-	3.81 s and 3.82 s	3.82 s	3.82 s	3.83 s

Spectra obtained in DMSO-d_6; peak **A**, **5-2**, **5-3**, **7**, **8-1**, and **8-2** were measured at 70 ºC. peak **9-1** was measured at 40 ºC.

Table 2. ^{13}C NMR Data of peaks **A**, **5-2**, **5-3**, **7**, **8-1**, **8-2**, and **9-1**

Carbon	Chemical shift δ in ppm						
	A	**5-2**	**5-3**	**7**	**8-1**	**8-2**	**9-1**
2	162.8 s	163.5 s	163.4 s	163.2 s	163.2 s	163.2 s	161.1 s
3	102.9 d	102.5 d	102.3 d	102.9 d	102.8 d	102.5 d	106.4 s
4	181.3 s	181.4 s	181.4 s	181.5 s	181.5 s	181.4 s	177.1 s
5	160.6 s	160.8 s	159.4 s	160.4 s	160.5 s	160.7 s	158.4 s
6	108.0 s	107.8 s	107.7 s	107.8 s	107.8 s	107.9 s	104.3 d
7	162.8 s	163.5 s	163.4 s	163.2 s	163.2 s	163.2 s	162.6 s
8	93.6 d	93.2 d	93.1 d	93.3 d	93.3 d	93.4 d	98.5 d
9	156.3 s	156.1 s	156.0 s	156.2 s	156.2 s	156.2 s	158.6 s
10	102.9 s	103.0 s	102.9 s	103.0 s	103.0 s	102.5 s	108.2 s
1'	121.5 s	121.5 s	121.4 s	121.6 s	121.6 s	121.2 s	120.4 s
2'	110.4 d	113.2 d	113.1 d	110.3 d	110.3 d	127.9 d	104.5 d
3'	147.9 s	145.4 s	145.3 s	147.7 s	147.7 s	115.5 d	148.2 s
4'	150.6 s	149.3 s	149.3 s	150.4 s	150.5 s	160.7 s	139.4s
5'	115.7 d	115.6 d	115.6 d	115.5 d	115.5 d	115.5 d	148.2 s
6'	120.0 d	118.6 d	118.6 d	120.1 d	120.1 d	127.9 d	104.5 d
1''	-	125.4 s	124.7 s	125.3 s	124.8 s	125.4 s	-
2''	-	111.1 d	129.7 d	111.0 d	129.6 d	111.1 d	-
3''	-	147.7 s	115.4 d	147.7 s	115.4 d	147.7 s	-
4''	-	149.0 s	159.4 s	149.0 s	159.4 s	149.1 s	-
5''	-	115.3 d	115.4 d	115.3 d	115.4 d	115.3 d	-
6''	-	122.5 d	129.7 d	122.4 d	129.6 d	122.5 d	-
α	-	114.0 d	113.5 d	113.9 d	113.7 d	114.0 d	-
β	-	144.1 d	143.9 d	144.1 d	143.8 d	144.1 d	-
-COOR	-	165.8 s	165.8 s	165.9 s	165.9 s	165.9 s	-
G-1	71.2 d	71.0 d	70.9 d	71.0 d	71.1 d	71.1 d	104.1 d
G-2	80.2 d	81.1 d	80.2 d	81.1 d	80.7 d	81.1 d	73.6 d
G-3	78.1 d	78.3 d	78.3 d	78.3 d	78.3 d	78.3 d	75.6 d
G-4	70.2 d	70.1 d	70.0 d	70.1 d	70.1 d	70.1 d	69.7 d
G-5	81.0 d	81.1 d	81.3 d	81.1 d	81.1 d	81.1 d	77.5 d
G-6	61.2 t	61.2 t	61.1 t	61.2 t	61.2 t	61.2 t	60.8 t
G-1'	104.6 d	105.1 d	104.9 d	105.1 d	105.0 d	105.0 d	-
G-2'	74.4 d	74.2 d	74.2 d	74.2 d	74.2 d	74.2 d	-
G-3'	76.3 d	76.2 d	76.1 d	76.3 d	76.3 d	76.3 d	-
G-4'	69.7 d	68.9 d	68.7 d	68.9 d	69.0 d	68.9 d	-
G-5'	76.0 d	73.2 d	73.1 d	73.2 d	73.3 d	73.3 d	-
G-6'	60.7 t	62.1 t	62.0 t	62.1 t	62.2 t	62.2 t	-
-OCH$_3$	55.9 q	55.6 q	-	55.7 q	55.7 q	55.6 q	56.3 q
-OCH$_3$	-		-	55.6 q	-	-	56.3 q

Spectra obtained in DMSO-d_6; peak **A**, **5-2**, **5-3**, **7**, **8-1**, and **8-2** were measured at 70 ℃. peak **9-1** was measured at 40 ℃.

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